UNIVERSITY OF ALBERTA

An Evaluation of Western Canadian Six-Row Barley (*Hordeum vulgare L.*) Cultivar Differences in Germination Rate and Seedling Growth Under Low Temperature Stress

By

Gordon J. Dunn

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

Master of Science

In

Agronomy

Department of Plant Science

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **An Evaluation of Western Canadian Six-Row Barley (*Hordeum vulgare L.*) Cultivar Differences in Germination Rate and Seedling Growth Under Low Temperature Stress** submitted by **GORDON J. DUNN** in partial fulfillment for the degree of **Master of Science** in **Agronomy**.

Dr. K. G. Briggs (Supervisor)

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Dr. J. A. Robertson

Date 9/3/92

I dedicate this thesis with love and appreciation to my father and mother, John and Ellen Dunn, and to my sisters, Marion Harry, Margaret Fournier, and Carol Barr without whose loving and patient support completion would not have been possible.

ABSTRACT

Barley cultivars with rapid germination rate and vigorous stand establishment characteristics would be desirable to producers using conservation tillage cropping systems that may at times necessitate the sowing of crops into cooler than normal seedbeds. The present study was conducted to evaluate a representative sample of western Canadian six-row barley cultivars for differences in germination rate and early seedling vigor under normal conditions and under low temperature stress conditions.

The fifteen barley cultivars selected for study include malt types, feed types, early to late maturing cultivars, and tall to semidwarf types. Growth chamber assays were conducted at a series of temperatures between 2.5°C and 20°C to determine relative differences among cultivars in germination rate and seedling growth rates. Field trials were conducted using the same cultivars seeded on a series of dates to assess cultivar emergence rates and seedling growth rates over a range of seedbed temperatures. Two respiration rate assay techniques, tetrazolium reduction and CO_2 evolution, were evaluated for their potential use as rapid screening procedures in breeding programs.

Germination rate was found to be significantly different among cultivars, and relative ranking of cultivars was consistent at all assay temperatures. Differences in seedling growth rate among cultivars, quantified as plant dry weight, were also consistent at all temperatures, but cultivar ranking for growth rate was not the same as for germination rate. Days to emergence in field trials were significantly correlated with data from germination rate assays. Seedling growth measurements in the field were generally correlated with seedling dry weights assayed in the growth chamber, but random cultivar variability in seedling growth was greater than for germination rate, consequently correlations were not always significant.

Both of the respiration assay techniques tested successfully differentiated cultivars on the basis of germination rate and field emergence rate. These procedures offer the advantage of rapid determinations with relatively low time and manpower expenditure, and could easily be adapted to screen large numbers of genotypes in breeding and testing programs. Cultivar specific information regarding germination rate would be of value to plant breeders as well as producers.

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PART I: INTRODUCTION

1.1: Background

In the prairie regions of western Canada the practices of zero-tillage and early seeding are gaining popularity with producers because of advantages gained through soil conservation, improved moisture utilization and opportunities for reduced production costs. This often results in the sowing of crops into colder and wetter than normal seed beds (Voorhees et al., 1981). In a study of the effects of soil thermal properties and surface residue cover on soil temperature under several tillage systems, Potter et al. (1985) observed reductions in soil temperature and soil heat flux at the 2.5 cm and 5 cm depths under zero tillage as compared to conventional tillage treatments. The reductions in temperature were attributed primarily to the presence of surface residue. McCalla and Army (1961) stated that soil temperatures are cooler beneath residue because of the insulating effects of the residue, reflection of radiant energy by the residue, and reduced evaporation due to the residue cover.

Stand establishment of spring sown cereal crops is a critical phase of crop production. The early emergence of a uniform and vigorously growing seedling population contributes positively to crop yield (Stoskopf, 1985). Seedlings are highly susceptible to soil-borne pathogens in the early stages of development, and rapid germination and emergence reduces the duration of exposure to infection. The impact on yield from foliar diseases may be reduced through earlier establishment because the crop will mature earlier and thus escape some of the impact of foliar disease epidemics occurring later in the season. Earlier crop maturity can also be an important advantage in northern regions where the number of frost-free days during the growing season is often a limiting factor in crop production. Competition from weeds can be significantly reduced by early development of a vigorous crop cover to

intercept moisture, nutrients and sunlight that otherwise may be absorbed by weed species. At low soil temperature germination and seedling emergence are delayed thereby partially counteracting the advantages of early seeding.

In western Canada, barley is an important field crop ranking second in acreage of production behind wheat (Anonymous, 1990) and is especially suitable for higher latitude regions where the growing season is shorter. A recent trend in the breeding of new six-row barley (*Hordeum vulgare* L.) cultivars for these regions is the development of high yielding semidwarf types. This is a result of the plant breeders' response to the producers' desire for cultivars that will utilize high moisture and nutrient inputs to increase grain yield, and at the same time resist lodging. In 1986 Samson, a semidwarf six-row feed barley cultivar, was registered for commercial production in western Canada. Because Samson is late maturing and has short coleoptile length, a common characteristic of semidwarf genotypes, producers were advised to seed as early as possible in the spring, and as shallow as possible depending on the depth to soil moisture (Helm, 1986).

A three year field study was conducted jointly by University of Alberta and Alberta Agriculture personnel to compare the performance of Samson to Leduc, a previously registered six-row feed barley cultivar, over a range of soil fertility levels at a number of locations in central Alberta (Briggs, 1988). Observations from these field plots, as well as reports from pedigreed seed producers, indicated that Samson exhibited poor stand establishment characteristics. Seedling emergence of Samson was obviously slower than Leduc under equivalent conditions, and early seedling growth seemed to lack the necessary vigor to compete with persistent weed species. However, the high yield potential and excellent lodging resistance of Samson were confirmed in the field trials (Briggs, 1991). Further breeding could potentially lead to the development of semidwarf cultivars with improved germination rate and seedling vigor compared to Samson. Genotypes capable of rapid germination and early

seedling growth at low temperatures would be desirable as parents in such breeding programs.

Recent studies have successfully identified differences in cold temperature growth ability among spring wheat genotypes (Johnston et. al., 1986; Lafond and Baker, 1986b). Similar genotypic differences in germination or growth rate have been reported for a number of crop species including corn (Gubbels, 1974), sorghum (De La Soujeule, 1985), rapeseed (Kondra et al., 1983), and soybean (Mortensen et al., 1986). Boyd et al. (1971) reported inherent differences in germination rate among F_3 progeny from several six-row and two-row barley crosses, and found significant ($P<0.01$) correlation between germination rate and seedling dry weight two weeks after germination. Since the study by Boyd et al. (1971) there have been no published reports concerning the existence and extent of variability in germination rate among six-row barley cultivars registered for production in western Canada. Further, there is a lack of published information that evaluates different methods of screening genotypes for differences in germination rate and seedling vigor.

1.2: Study Objectives

The objectives of the present study are threefold:

(1) to quantify differences in germination rate and seedling growth rate among western Canadian six-row barley cultivars over a range of temperatures;

(2) to compare results from laboratory and growth chamber screening methods to field trial assessments of emergence rate and seedling growth of six-row barley genotypes subjected to low temperature stress;

(3) to evaluate respiration assay techniques for effectiveness in screening six-row barley genotypes for tolerance to low temperature stress during germination and early seedling growth.

PART II: LITERATURE REVIEW

2.1: Low Temperature Effects on Plant Growth and Development

Plant development is primarily a function of cellular biochemical reactions under enzymatic control, and the rate of reactions and integrity of enzymes are very much dependant upon temperature. Plant metabolic processes, such as respiration rate, generally display a temperature quotient (Q_{10}) near 2 in the 5 to 25°C temperature range (Salisbury and Ross, 1978). For example, each temperature increase of 10°C will double respiration rate. Hence, all the physiological processes dependant on the energy (ATP) generated by respiration are affected by variations in temperature.

Plants exposed to low temperatures suffer from reduced reaction rates and a number of other physical consequences, such as increased viscosity of protoplasm, decreased vapor pressures, and loss of fluidity in cell membranes. The combined effects of low temperature on plants is often referred to in the literature as chilling stress, which in general refers to temperatures below 10-15°C and down to 0°C (Levitt, 1980).

Lyons and Raison (1970) proposed that the primary event causing chilling injury to plants is a physical phase transition of cellular membranes from a liquid-crystalline to a solid-gel state. This hypothesis was developed from observing that the respiratory activity of mitochondria isolated from chill-sensitive plants (sweet potato, tomato, and cucumber) exhibited a normal Q_{10} decrease as temperature decreased, but an abrupt change in respiration rate decrease occurred near the temperature critical for development of chilling injury symptoms in whole plants. The authors concluded that reduced respiratory activity due to disruption of mitochondrial membranes is a primary response of plants to low temperature.

The phase transition of cellular membranes is proposed to be a result of a change in the degree of molecular ordering of membrane lipids, bringing about two immediate effects: (1) an increase in membrane permeability causing rapid water loss and increased ion leakage from cells, and (2) an increase in activation energy of membrane bound enzymes due to enzyme conformational changes imposed by the transition of membranes to the solid-gel state (Lyons, 1973). Subsequent disruption of cell function as a result of the primary membrane phase change response is credited with the diverse range of chilling injury effects. It has been proposed that continued exposure to chilling stress leads to a number of harmful events, including loss of membrane integrity, leakage of solutes and ions across cellular and subcellular membranes, decrease in mitochondrial respiration, reduction in ATP production, cessation of protoplasmic streaming, decrease in photosynthetic rate due to thylakoid destruction, metabolic dysfunction due to ion imbalance, accumulation of toxic metabolites, and disruption of cell cytoskeleton structure (Wang, 1982). Using electron-spin resonance spectroscopy, Raison et al. (1971) determined that mitochondrial membrane phase transition coincided with the temperature at which the chilling injury occurred in several plant species, and that changes in the activation energy of membrane associated enzymes occurred at this same critical temperature. Wade et al. (1974), also using spin resonance techniques, detected the occurrence of a phase transition, as indicated by Arrhenius plot breaks, at about 10°C in mitochondria, glyoxysome, and proplastid membranes of castor bean.

Various injury symptoms caused by chill stress have been described for plants depending on species, severity and duration of exposure to low temperature, and other environmental conditions such as light intensity, relative humidity, soil moisture status and nutrient availability. In many species the first observable symptom is leaf wilting, followed by the development of sunken, water-soaked pits which turn to brown necrotic patches with prolonged exposure to chilling or on return of plants to

warmer temperature (Lyons, 1973). Most chill sensitive crop species (e.g. corn, rice, field bean, tomato, cotton) suffer stress when exposed to temperatures below 15°C and will show visible signs of injury after 24 to 48 hours at 5°C (Wilson, 1984). Chilling tolerant species (e.g. wheat, rye, barley, pea) can withstand prolonged exposure to temperatures in the 0°C to 5°C range without obvious injury, but growth rate is greatly reduced and plants are often stunted due to restriction of cell expansion and elongation (Andrews, 1987). Visible symptoms of chilling injury to roots are mainly root browning and the development of stelar lesions near the root tip (Wilson, 1984).

2.1.1 Effects on Photosynthesis

Photosynthesis is reduced by chilling temperatures, and the impairment of photosynthesis is increased if the chilling occurs in the presence of light or if the plant is water stressed (Martin et al., 1981). Stomatal closure due to chilling-induced water stress is responsible for part of the decrease in photosynthesis, but direct chloroplast injury has also been reported (Wise and Naylor, 1987). Chill stress in the presence of high light intensity causes rapid chlorophyll destruction in some species and chlorosis of upper leaves can occur within 24 hours of the onset of low temperature exposure (VanHasselt and Strikwerda, 1976). The precise location of this injury, however, is not known. Decreased fluorescence from intact tissue or isolated chloroplasts and reduction in quantum yield of whole-plant photosynthesis suggests that the oxidative side of photosystem II is the site of injury (Powels et al., 1983; Smillie and Hetherington, 1983).

In barley, a chill-tolerant species, photosynthetic abnormalities have been noted in plants grown at 5°C (Smillie et al., 1978). Fewer than normal chloroplasts developed and thylakoids displayed crimped-looking membrane deformities. The actively growing portions of the leaves (intercalary meristem) were permanently affected when chill stress was applied, and did not become green on return to warm

temperatures, resulting in chlorotic bands around the mature leaves. Singh and Singhal (1985) investigated the effects of low temperature (8°C) and high temperature (45°C) stress on barley seedling chloroplasts at different stages of development (12, 48 and 72 h). Changes in light absorbance were induced by both temperature extremes, and the reversibility of absorbance change was maximum in chloroplasts greened for 72 h and minimum in chloroplasts greened for only 12 h, indicating that fully developed chloroplasts have greater capacity to recover from temperature stress. Most manifestations of chilling injury are reversible if stress duration is short, but long-lasting chill stress causes permanent damage to photosynthetic apparatus and other organelles, resulting in significantly decreased crop yield (Wilson, 1984). Even short periods of chilling, such as overnight, have been shown to cause depressed photosynthetic rates in fieldbean for several days after return to warmer conditions (Crookston et al., 1974).

2.1.2 Effects on Root Growth and Function

Root growth and function is slowed at low temperatures. Studying root growth of wheat seedlings, Jordan et al. (1985) measured Q_{10} values near 2.0 in the 10 to 20°C range, but below 10°C the Q_{10} values ranged from 4.3 to 10.5 indicating a larger reduction in activity with decreased temperature than would be expected from a normal Q_{10} response. Root systems of barley plants grown at 5 and 10°C were shorter and thicker with less secondary root development than plants grown at 20 and 25°C (Abbas Al-Ani and Hay, 1983; Schwartz et al., 1987). Lowering root media temperature from 14°C to 5°C caused a reduction in nitrate and ammonium ion uptake rate per unit of root fresh weight, per unit of root surface area, and per unit of root length (MacDuff and Hopper, 1986). Even though complete phase transition from liquid to gel state of cell membranes of chill-tolerant species such as wheat and barley is reported to occur at temperatures near 0°C (Pike et al., 1979), recent studies (Caldwell

and Haig, 1981; Caldwell and Whitman, 1987) have detected the presence of quasicrystalline lipid clusters in the membranes of barley root cells at 7 to 12°C. These clusters may influence the activity of membrane-bound proteins and adversely affect normal root function.

2.1.3 Effects on Germination

Seeds planted into cool soil often exhibit reduced germination percent and germination rate and are more susceptible to pathogenic infection (Miedema, 1982; Lafond and Baker, 1986b). Cardinal temperatures for germination of temperate climate cereal crops, such as wheat and barley, are reported as: minimum, 3 to 5°C; optimum, 20 to 25°C; maximum, 35 to 40°C (Peterson, 1965; Evans et al., 1974). Variability in cardinal temperatures can be expected dependant upon species, cultivar and source of different seed lots. It has long been known that small grains, including barley, are capable of germination at temperatures near 0°C (Coffman, 1923) but, at temperatures this low, germination rate is so slow that successful establishment of a viable seedling under field conditions is doubtful.

Germination involves a series of physical and biochemical processes from the initial imbibition of water and the re-establishment of membrane function to the activation of numerous enzymes and metabolic pathways, and ultimately the elongation of the root initial through the testa. Simon et al. (1976) stated that it is not easy to identify the particular point at which low temperature impedes germination. It is likely that low temperature affects all stages of germination to varying degrees depending on the species under study. The process of embryo and organelle re-hydration during seed imbibition is a very disruptive process and it has been suggested that species with high chill-tolerance in the vegetative stage may be very chill-sensitive during imbibition and growth initiation (Maguire, 1972). Obendorf and Hobbs (1970) reported that low temperature stress applied during imbibition of

soybean seeds reduced seedling survival and growth in the field. Pollock and Toole (1966) determined that the imbibition period was the critical temperature-sensitive stage in the germination of lima beans during which low temperatures caused reduced germination success and greater numbers of abnormal seedlings.

Lyons (1973) proposed that the physical state of cell and organelle membranes is responsible for chilling damage to imbibing seeds. A high degree of hydrogen saturation of fatty acids in membranes increases the critical temperature at which lipid phase transition from liquid to gel occurs. At low temperatures highly saturated membranes are less flexible and may suffer damage while swelling during imbibition. Membrane damage in turn disrupts metabolic processes, such as enzyme activity and solute translocation, resulting in less efficient utilization of seed stored energy reserves and, consequently, less vigorous seedlings (Maguire, 1972). Studies comparing the extent of membrane lipid saturation among species or cultivars differing in chill stress tolerance have revealed a general negative correlation between degree of saturation and chill tolerance (Markhart, 1986; Murata and Yamaya, 1984). However, conflicting evidence to this hypothesis has been reported (Wilson, 1984). It is probable that other germination factors, such as oxygen and water availability to metabolically active sites within the seed, interact with temperature and confound observable responses.

In summary, the points of importance to the present study are: (1) low temperatures during imbibition and early seedling development can cause damage to metabolic functions resulting in the development of less vigorous seedlings; (2) tolerance to low temperature stress varies among species and cultivars and is, to some degree, a heritable trait. These points may be significant when considering the importance of developing new crop cultivars with improved low temperature growth ability.

2.2: Seed and Seedling Vigor

The term 'vigor', when applied to seeds or seedlots, is very subjective and warrants a brief definition here. Perry (1972) provides a concise definition as follows: "A physiological property determined by the genotype and modified by the environment, which governs the ability of a seed to produce a seedling rapidly in soil, and the extent to which that seed tolerates a range of environmental factors". This is an accurate, but very simplistic, description of seed vigor which is obviously a highly complex property. Woodstock (1973) states that vigor at the biochemical level involves energy and biosynthetic metabolism, coordination of cellular activities, and transportation and utilization of reserve foods. At the level of seed germination it involves the speed and totality of germination and the range of environmental conditions under which germination will occur. Besides genetic factors, the vigor of a seed is affected by the environmental and developmental conditions under which it was grown, matured, harvested, and stored.

Vigor, in regard to seedlings and developing plants, generally refers to the plant's growth and development ability under the limitations imposed by environmental factors such as temperature, moisture, nutrients, sunlight, pathogens, and inter-plant competition. Seedling vigor is most often assessed using some sort of growth rate or developmental stage index. Dry matter accumulation, plant height, root length, and leaf extension rate are examples of commonly used growth rate indices. Time to emergence and new leaf appearance are examples of developmental stage indices.

2.2.1 Seed Dormancy and Ageing

Seeds of most cereal crop species undergo a period of dormancy for several months after maturation which diminishes with time to a stage where, under

optimum conditions, a seed lot's maximum potential for germination rate and germination percent can be realized (Gordon, 1972). As time progresses from this stage, seed vigor declines to the point of non-viability where germination will not occur under any conditions. Heydecker (1969), in reviewing the problem of assessing vigor in seeds, points out the need to distinguish between part-dormant and non-vigorous seeds, both of which will germinate slowly.

The length of time required for total loss of dormancy, as well as the length of time to the onset of vigor decline caused by ageing, is affected by both genetic and environmental factors. Gordon (1972) tested the germination percent and germination rate of seed lots of a number of cereal crop species, including barley, immediately after harvest and at monthly intervals thereafter for one year. Germination percent for most seed lots reached near maximum after 2 to 3 months. However, germination rate was still increasing for most seed lots after 5 months and for a number of seed lots after 10 months. Further, it was noted that although germination percent remained constant, germination rate of some seed lots began to decline near the end of the 12 month period. If germination rate is included as a component of seed vigor then clearly the standard germination test, which only assesses germination percent, is not an adequate measure of seed vigor.

2.2.2 Seed Source

The vigor of a seed can be attributed to a combination of genetic factors and environmental conditions under which the seed developed. Seed size, protein content and germination rate are all important components of seed vigor that have been shown to directly affect the vigor of subsequently developing seedlings, and all are subject to both genetic and environmental influence (Heydecker, 1969). In order to select for genetic improvements in seed vigor, the plant breeder must, as much as possible, maintain uniformity in environmental conditions under which seeds of

different genotypes are produced and assayed so that genetic expression of vigor is maximized. Gordon (1972) assayed the germination rate of 16 six-row barley cultivars from each of six production sites across Canada. Significant differences ($P<0.01$) were found among cultivars and among sites, as well as a significant cultivar by site interaction. The relative ranking of cultivars was fairly constant over sites and the significant site by cultivar interaction was attributed primarily to two cultivars. Lafond and Baker (1986a) found seed size to affect field emergence rate of wheat and recommended that seeds of similar size and from a common seed source (i.e. produced at the same time and in the same location) be used when comparing cultivars for inherent seed vigor differences.

2.2.3 Seed Size and Seed Protein Content

Seed size and seed protein content have been reported to affect barley seedling vigor as well as grain yield. Kaufmann and Guitard (1967) found a positive correlation between seed size, seedling vigor and final yield in barley. Boyd et al. (1971), also working with barley genotypes, attributed variability in seedling vigor to seed size and germination resistance. With wheat, Kittock and Law (1968) found that seed weight correlated positively with emergence rate and shoot dry weight in greenhouse experiments, indicating that smaller seeds tend to produce less vigorous seedlings. Evans and Bhatt (1977) showed that wheat seed size was positively correlated to seedling dry weight 20 days after germination. They also noted that in their study percentage protein was greater in large seeds. However, Austenson and Walton (1970), studying wheat cultivars, reported that size of the individual seeds accounted for only 2.5% to 4.5% of variation in final yield.

The increased vigor of large seeds has been attributed to greater quantities of endosperm material, which is utilized during the pre-photosynthetic stage of seedling development. Bremner et al. (1963) found that the seed embryo size had negligible

effect on seedling vigor in wheat, while the effect of endosperm size was considerable. McDaniel (1969) reported that superior seedling vigor obtained from heavier barley seeds was related to increased quantities of mitochondrial protein and higher respiratory activity of seed mitochondria. Increased mitochondrial respiration activity is assumed to lead to a higher respiratory rate and greater amounts of energy (ATP) production, thus giving heavier seeds a greater growth rate potential than lighter seeds.

Lowe et al. (1972a) and Ries and Everson (1973) also found that seedling vigor and grain yield of wheat were associated with seed size and seed protein content. The best relationships were obtained between the amount of protein per seed and seedling vigor indicating the possibility that, to some extent, larger seeds produced more vigorous seedlings because they contain more total protein. Lowe and Ries (1972b) and Bulisani and Warner (1980) reported that small wheat seeds with high percent protein produced seedlings with superior dry matter accumulation compared to larger seeds of the same genotype with lower percent protein. Metivier and Dale (1977) found that barley cultivars with high seed protein emerged faster and produced more seedling dry matter compared to cultivars lower in seed protein.

Lopez and Grabe (1973) reported increased speed of emergence and higher dry matter accumulation of seedlings from high protein seeds grown in nitrogen deficient soil. However, Ries et al. (1970) and Bulisiani and Warner (1980) have shown that if sufficient exogenous nitrogen is available to the seeds at the time of germination and early seedling growth, the differences in seedling dry matter accumulation between seeds of high and low protein content disappear. They conclude that adequate nitrogen supply is probably the most important factor governing the expression of seedling vigor as affected by seed protein content. Further, they state that if enough nitrogen is available to the seed within the first three days of germination, the effects

of seed protein content are lessened or eliminated, while under less fertile soil conditions, the higher protein seeds will develop into seedlings with superior vigor.

2.3: Screening Genotypes For Low Temperature Vigor

Numerous tests and indices have been developed for the purpose of assessing seed and plant vigor, and they all have one thing in common - none are perfect. The complex nature of the vigor concept precludes the existence of a single test that will accurately evaluate, and derive a quantifiable value, for all vigor related components. Vigor is composed of a number of plant characteristics often interacting with one another and with environmental conditions. Heydecker (1960) stated that climatic patterns impose different sets of unfavorable conditions in different seasons and areas, thus no one vigor test can be expected to be representative of all possible sub-optimal conditions seeds will encounter when sown in the field. Therefore, the first steps in screening genotypes for vigor differences must be to identify the components of vigor that contribute the most to the overall vigor of the plants under expected field conditions and then to determine the test or tests that will best evaluate the specific vigor components of interest. The ideal vigor test should be rapid, easy to perform, and able to detect small as well as large differences (Copeland, 1985).

Tests for the evaluation of seed or plant vigor at low temperature can be classified into three categories: (1) evaluation of growth and development rates at low temperatures in growth chambers; (2) evaluation of biochemical processes that are directly or indirectly related to growth rate under low temperature stress; (3) evaluation of growth and development rate in the field. Growth tests in growth cabinets offer the advantages of closely controlled and reproducible environmental conditions so that the effects of specific stresses can be measured. Tests that quantify specific biochemical processes within plants subjected to low temperature stress are

desirable as indices because such tests are often faster and require less equipment than growth tests. They often have the added advantage of providing more detailed information concerning the cellular and subcellular nature of low temperature stress response which may be important in the genetic engineering of tolerance gene complexes. A major disadvantage of biochemical indices is that they fail to measure whole plant responses which are likely influenced by a number of interactive components (Woodstock, 1973). Field trials are important for assessing low temperature vigor since it is impossible to exactly duplicate field conditions in the growth cabinet. The accuracy of tests performed under simulated conditions is commonly assessed by correlation to field results. However, field conditions are difficult to predict and data from several years is necessary for accurate assessment.

2.3.1 Growth Chamber Screening

Germination percent, measured under near optimum conditions, is the most common test that is routinely performed on seed lots. Under favorable field conditions close correlation between germination test results and field emergence of cereal crops can be expected, but this correlation is often lost when the seeds encounter increased environmental stress such as low soil temperature (Don et al., 1981). For this reason the 'cold test' (Wiesmer, 1988), which is routinely used on corn seed lots, was developed to evaluate germination percent under simulated field conditions. Seeds are germinated at 10°C in soil moistened to field capacity, and counts of the number of normal seedlings that develop are taken after 11 days.

Germination rate is a preferred indicator of seed vigor at low temperature because it can detect small differences in growth rate that can be significant to successful stand establishment in the field (Gordon, 1972). Germination rate, in a statistical context, is a measurement of a population mean (e.g. the mean of 100 seeds per experimental unit) and analysis of data should take this into account. In a review

of data analysis methods for seed germination, Scott et al. (1984) state that standard analysis of variance or regression procedures are appropriate where germination of all viable seeds is achieved, but inappropriate when some viable seeds fail to germinate. Gordon's (1971) germination resistance (rate) procedure takes this into account by using the total number of seeds germinated as the divisor in the equation for calculating mean germination time of a treatment.

Seedling growth tests at low temperature in a growth cabinet are commonly used to assess vigor because a high degree of reproducibility and accuracy in detecting genotypic differences is possible (Woodstock, 1973). Growth rate can be measured in a number of ways, including dry matter accumulation of roots and shoots at time intervals, length of roots and shoots, leaf extension rate, and new leaf appearance rate (Perry, 1976). With cereal crop species, measurements that assess total plant growth (e.g. dry matter accumulation) are likely to be more indicative of vigor than measurements of height or lengths when comparing cultivars because of inherent differences in plant architecture that may confound the results.

2.3.2 Biochemical Indices for Screening

The numerous biochemical assay procedures that have been investigated for use as indices for vigor testing were critically reviewed by Woodstock (1973). In his view, the most useful process to measure will: (1) be fundamental and essential in germination; (2) involve the coordinated activity of many enzymes thereby serving as a measure of general metabolic activity; (3) be relatively easy to measure. Respiration rate meets all these requirements and has the advantage that assay techniques can be adapted to tests of seed lots, or for measurements on single seeds.

Respiration rate can be simply quantified by either measuring oxygen uptake or carbon dioxide evolution. Woodstock and Grabe (1967) reported a correlation of $r=0.96$ between oxygen uptake of imbibed barley seeds and shoot length of five-day old

seedlings. They were able to detect respiration differences in seed lots caused by stress treatments such as freezing, heating, chilling, and gamma-irradiaton. Kittock and Law (1968) found a significant positive correlation between respiration of germinating wheat seeds and seedling dry weights. With cotton seed, Bishnoi (1971) reported that respiration and cold test results were better correlated with stand establishment than were visual tetrazolium staining ratings. Krishnasamy and Seshu (1989) assayed 11 rice cultivars using a number of physiological and biochemical parameters of seeds and tested their results for correlation to germination rate. Through path correlation analysis, oxygen uptake and dehydrogenase activity were found to contribute the most to germination rate variability.

Briggs and Horak (1980) compared the ATP content of dry seeds of 75 spring wheat cultivars to assays of germination rate in growth cabinets and seedling dry weights from field plots. They found significant genotypic differences for all variables measured but no relationship was found between ATP content and germination rate or dry matter accumulation. Hall and Wiesner (1990) tested the effectiveness of several seed vigor tests in predicting speed of emergence, total emergence and forage yield of meadow bromegrass. Only total emergence was correlated with standard germination tests, while both total emergence and forage yield were correlated with accelerated ageing treatments, respiration measurements and ATP assays. They concluded that respiration rate and accelerated ageing are the best models for predicting first-year forage yield.

Another procedure that meets Woodstock's (1973) criteria for a seed vigor test is tetrazolium staining, which has been widely used for evaluating seed viability and quality (Moore, 1972). The reaction of tetrazolium molecules with H^+ ions released by the dehydrogenase enzymes involved in the respiration of living tissues results in the production of formazan, an oil-soluble red pigment. The extent and location of

formazan accumulation within the tissues of seeds imbibed in a weak solution of tetrazolium chloride can be visually assessed and used to rate seed vigor of large seed species like corn or field beans (Grabe, 1976). Small seed species, such as barley, can be evaluated for viability but their seeds are too small to be dissected and inspected for embryo soundness as is done with corn. Also, the vigor rating obtained through this method is highly dependant on operator skill and judgement and is not quantitative. A variation of the tetrazolium test has been proposed and tested (Kittock and Law, 1968; Johnston et al., 1986) in which the formazan is extracted with solvent from the seed embryo tissue and the amount from each treatment sample is quantified using optical density measurements. Johnston et al.(1986) were able to detect reproducible wheat cultivar differences in low temperature vigor using this method, and reported good correlation between optical density readings and seedling dry matter accumulation at 10°C. Sung and Chen (1988) used this same technique to test rice cultivars for differences in seedling vigor over a range of temperatures from 10°C to 30°C and obtained significant high correlations (e.g. r=0.90 at 10°C) between optical density readings and seedling dry weights at all temperatures. The promising results obtained in previous studies that have tested the relationship between quantitative assays of tetrazolium reduction via seed respiration and seedling vigor assays indicate that further evaluation of this technique is warranted.

PART III: MATERIALS AND METHODS

The study was divided into three groups of experiments designed to assess cultivar differences in germination rate and seedling vigor under growth chamber, field and biochemical assay conditions. The seed lots used were harvested from regional barley cultivar trials grown at the University of Alberta Ellerslie Research Station in 1985 (seed source 1) and 1986 (seed source 2). Sufficient seed quantity of 15 six-row barley cultivars was available from both years to allow the use of the same two seed sources in all experiments. Brief agronomic descriptions of the cultivars used in the study are included in Table 2.1. The two numbered cultivars were tested in regional trials but have not been registered.

All seed lots were screened sequentially through #6 (3.4 mm) and #7 (2.9 mm) Canadian Standard Testing Sieves to remove the extreme large and small or broken seeds, approximately 15-20% of the seed lot. Two samples of 100 seeds from each seed lot were germinated at 20°C to determine viability. All samples germinated over 95%.

3.1: Experiment 1. Growth Chamber Trials

3.1.1 Germination Resistance

The germination resistance assays were conducted in a temperature and humidity controlled germination cabinet. Four samples of 100 seeds each were taken from each seed lot and placed on moistened filter paper in plastic trays. The trays were arranged in the germination cabinet in a randomized complete block design, with each block on a separate shelf in the cabinet. Each block contained samples of the 15 cultivars from each of the 2 seed sources, totalling 30 experimental units per block. The assays were repeated at six temperatures; 2.5, 5, 7.5, 10, 15, and 20°C. Counts of germinated seeds were taken at 24 hour intervals (12 hour intervals for 15

Table 2.1: Kernel weights (mg) and descriptions of the 15 six-row barley cultivars used in all experiments.

	Average Kernel Weight [1]		Average Height (cm) [2]	Relative Maturity [3]	Eligible Grades [3]
	Seed Source 1	Seed Source 2			
Argyle	36.6	35.6	90	medium	malt
Bonanza	35.4	36.5	88	medium	malt
BT 518 [4]	34.0	36.5	79		
BT 522 [4]	40.7	41.5	84		
Conquest	36.5	34.7	90	medium	malt
Diamond	39.5	38.8	75	medium	feed
Empress	36.3	34.7	81	medium	feed
Galt	35.9	34.1	75	medium	feed
Heartland	34.5	35.5	70	medium	feed
Jackson	36.1	35.6	67	early	feed
Johnston	34.4	35.1	89	late	feed
Klondike	34.4	36.5	79	medium	feed
Leduc	38.1	36.2	78	medium	feed
Otal	32.4	33.8	85	early	feed
Samson [5]	36.3	34.5	72	late	feed
Standard Error	0.93	0.87			

[1] Means of four samples, 500 kernels per sample, determined after sieving.
[2] Means of 1985 and 1986 Regional Cultivar Trials at Ellerslie.
[3] Source: Varieties of Cereal and Oilseed Crops for Alberta - 1987. Alberta Agriculture (Agdex 100/32).
[4] Regional cultivar trial entries that were not subsequently registered.
[5] Semidwarf cultivar.

and 20°C assays). Germination was considered to have occurred when the primary rootlet could clearly be seen protruding through the coleorhiza at the base of the seed. After each count the trays were re-randomized within blocks. Mean time to germination, termed germination resistance (GR) by Gordon (1971), was calculated for each experimental unit using the formula:

$$GR = \frac{\frac{t1}{2}(n1) + \frac{t1+t2}{2}(n2-n1) + \ldots + \frac{ti+tj}{2}(nj-ni)}{n(total)}$$

t = time in hours from beginning of experiment
n = number of germinated seeds counted

3.1.2 Seedling Total Dry Weight

Seedling growth tests were conducted in controlled environment growth chambers at five temperatures: 5, 7.5, 10, 15, and 20°C. Fifteen seeds of each cultivar from both seed sources were planted in six inch diameter pots filled with a mixture of soil-peat-perlite and fertilized with an aqueous solution of 20-20-20 fertilizer. The seeds were hand selected to be as uniform in size and soundness as possible. Water was added to water holding capacity of the soil mixture and the pots were placed in the growth chambers in randomized complete blocks, one block (replication) in each of the four quadrants of a cabinet. After emergence the seedlings were thinned to ten plants per pot to ensure equal plant density. The plants were watered as required and pots were re-randomized within blocks every two or three days. When the majority of plants reached the 4 to 5 leaf stage all pots were removed from the cabinet and the plants, including roots, were harvested, oven dried and weighed on a per pot basis.

3.2: Experiment 2. Field Trials

Field plots were established in 1987 and 1988 at two locations per year, on the Edmonton and Ellerslie Research Stations of the University of Alberta. Plots at both locations were on level, well drained Black Chernozemic soil that had been summerfallowed the previous year. Prior to seeding 90 kg ha^{-1} of 11-55-0 and 40 kg ha^{-1} of 0-0-44-18(S) fertilizers was broadcast and tilled in to ensure an abundance of macro nutrients. The plots were arranged in a 4 replicate split-plot randomized complete block design with 3 seeding dates as main plots and 15 cultivars as subplots. Seed source 1 was used to establish 1987 plots and seed source 2 was used to establish 1988 field plots. Each treatment consisted of 4 rows, 6 m in length, with rows spaced 23 cm apart. Seeding rate was adjusted for each cultivar to provide an expected plant density of 250 plants m^{-2}. Seeding dates were chosen to provide as wide a range of soil temperatures as possible among the three seeding dates. Immediately after seeding, soil temperature probes were buried at seeding depth (4 cm). The temperature probes were connected to Campbell Scientific CR21 data loggers and hourly soil temperature readings were recorded on magnetic tape throughout the growing season.

3.2.1 Seedling Emergence Rate

Once seedling emergence began, seedling counts were taken daily from 1 m lengths of two rows in each treatment. Counts were terminated when treatments reached 75% of the expected plant population (calculated from the 250 plants m^{-2} seeding rate) and the data were recorded as days to 75% emergence.

3.2.2 Shoot Dry Weight

To assess treatment differences in seedling dry matter accumulation, ten plants per treatment were harvested at ground level three weeks and five weeks after seeding. The plants were oven dried and weighed, and the data were recorded as grams shoot dry weight per ten plants.

3.3: Experiment 3. Respiration Assays of Germinating Seeds

Two methods of assaying the respiration rate of germinating seed samples were tested to evaluate their effectiveness in detecting differences in germination rate among cultivars: (1) carbon dioxide (CO_2) concentration measured in gas samples drawn from sealed flasks containing germinating seed samples; (2) reduction, via seed metabolism, of tetrazolium chloride (TZ) to formazan. The formazan, a red colored compound, was extracted from germinating seeds with solvent and quantified using optical density measurements. These assays were conducted at four temperatures: 5, 10, 15, and 20°C.

3.3.1 Carbon Dioxide Evolution

Twenty-five seeds were selected from each seed lot and placed in 25 ml Erlenmyer flasks. Distilled water (1 ml) was added and the flasks were placed in temperature controlled cabinets for 48 hours for the 5 and 10°C assays and 24 hours for the 15 and 20°C assays. Preliminary tests indicated these times to be adequate for the majority of seeds to imbibe water and start to germinate (i.e. to reach chitting stage). At this stage the number of seeds per flask was reduced to 20 viable seeds, and the atmosphere in each flask was displaced with compressed air. The flasks were then sealed with rubber septums and replaced in the cabinets. Gas samples (1 ml)

were extracted after 2 and 4 hours of the flasks being sealed, and injected into a gas chromatograph for CO_2 analysis. The exact volume of each flask had been determined by weighing the flasks empty and filled with water then taking the difference, assuming 1 ml of distilled water equals 1 gram. Volume correction factors (calculated volume/25 ml) were calculated and applied to the CO_2 readings from each sample. A control flask containing 1 ml water but no seeds was included with each replication. The CO_2 readings from the control flasks were subtracted from the sample CO_2 measurements to determine the amount of CO_2 evolved from seed respiration. The respiratory CO_2 amounts were divided by the time intervals before gas sampling (2 and 4 h) to determine the CO_2 evolved per hour ($\mu mol\ h^{-1}$), and the 2 and 4 hour samples were averaged together. After the TZ reduction experiment was completed the seeds were oven dried and weighed. The calculated CO_2 $\mu mol\ h^{-1}$ were then divided by the seed sample dry weights to derive the evolved CO_2 $\mu mol\ h^{-1}\ g^{-1}$. Each assay was replicated four times.

3.3.2 Tetrazolium Chloride Reduction

To measure the TZ reduction the seeds from the CO_2 experiment were removed from the flasks after the last gas sample was drawn, tied in gauze pouches and placed in beakers containing a solution of 0.2% TZ and distilled water (Johnston et al., 1986). The beakers were placed in the temperature controlled cabinets for 24 hours for the 5 and 10°C assays and 12 hours for the 15 and 20°C assays with air bubbling through the solution. The seeds were then removed from the TZ solution, rinsed with distilled water and placed in vials containing 5 ml of methylcellosolve for 24 hours at room temperature to extract the formazan. Optical densities of the solvent-formazan mixtures were measured at 480 µm using a spectrophotometer (Baush and Lomb Spec 21). A standard curve was generated using known dilutions of reagent formazan and a formula was developed from this curve to convert optical measurements to

$\mu mol\ mol^{-1}$ of formazan in solvent. The calculated $\mu mol\ mol^{-1}$ values were divided by seed sample dry weights to determine $\mu mol\ mol^{-1}\ g^{-1}$ of formazan produced.

3.4: Statistical Analysis

Analysis of variance was performed on data from the germination resistance, seedling growth and respiration experiments using the model outlined in Table 2.2, and on the field plot data using the standard model for split-plot analysis (Sokal and Rohlf, 1980). All significance tests were performed at the 95% confidence level (P=0.05). Hartley's F_{max} test for homogeneity of variances (Sokal and Rohlf, 1980) indicated significant heterogeneity of variance from growth chamber and respiration assay data, due primarily to the temperature treatments. Data from these experiments were transformed to their common logarithms, and the analysis of variance and F_{max} tests were recalculated using the transformed data. Heterogeneity of variance among temperature means was reduced by data transformation but was still significant, and the significance of all F tests remained the same (at P=0.05) as with the original data. Consequently, to test for differences among cultivars within each assay temperature and seed source, one-way analysis of variance was performed on each set of 15 cultivars and the resulting standard errors were used to calculate Tukey's honestly significant difference (HSD) intervals. Pearson product-moment correlation coefficients (r) were calculated to test relationships among assay methods (Sokal and Rohlf, 1980).

Table 2.2: Analysis of variance model for germination resistance, seedling growth and respiration experiments. Temperature, cultivar, and seed source are treated as fixed factors. Replications are random and nested within temperature.

Source of Variation	Degrees of Freedom	Expected Mean Squares	Error Term for F Test
Temperature (T)	t-1	$\sigma^2_{R(T)} + c\,s\,r\,\phi_T$	R(T)
Replicate within T (R(T))	t(r-1)	$\sigma^2_{R(T)}$	
Cultivar (C)	c-1	$\sigma^2_{CR(T)} + t\,s\,r\,\phi_C$	C x R(T)
C x T	(c-1)(t-1)	$\sigma^2_{CR(T)} + s\,r\,\phi_{CT}$	C x R(T)
C x R(T)	(c-1)t(r-1)	$\sigma^2_{CR(T)}$	
Seed Source (S)	(s-1)	$\sigma^2_{SR(T)} + t\,c\,r\,\phi_S$	S x R(T)
S x T	(s-1)(t-1)	$\sigma^2_{SR(T)} + c\,r\,\phi_{ST}$	S x R(T)
S x R(T)	(s-1)t(r-1)	$\sigma^2_{SR(T)}$	
S x C	(s-1)(c-1)	$\sigma^2_{SCR(T)} + t\,r\,\phi_{SC}$	S x C x R(T)
S x C x T	(s-1)(c-1)(t-1)	$\sigma^2_{SCR(T)} + r\,\phi_{SCT}$	S x C x R(T)
S x C x R(T)	(s-1)(c-1)t(r-1)	$\sigma^2_{SCR(T)}$	
Total	tcsr - 1		

PART IV: RESULTS AND DISCUSSION

The three sets of experiments included in this study test cultivar differences in low temperature vigor under field, growth chamber and biochemical assay conditions. Growth chamber procedures similar to those used in Experiment 1 to measure germination resistance and seedling growth rate have been used previously by researchers working with cereal crop species (Gordon, 1972; TeKrony and Egli, 1991). Under the controlled conditions of growth chambers results tend to be highly reproducible. In this study, Experiment 1 results are used to determine the extent of differences in low temperature vigor among the 15 barley cultivars assayed (Objective 1). The Experiment 2 field trials were included in the study to allow comparison of growth chamber and biochemical assay results to field growing conditions similar to those encountered in commercial barley production (Objective 2). Respiration assays similar to the CO_2 evolution and tetrazolium chloride reduction assays tested in Experiment 3 have been used to estimate low temperature vigor in other crop species (Johnston et. al., 1986; Woodstock, 1973), but have not been evaluated using barley. Experiment 3 results are compared to growth chamber and field trial results to test the effectiveness of these procedures in screening barley genotypes for tolerance to low temperature stress during germination (Objective 3).

4.1: Experiment 1. Growth Chamber Trials

4.1.1 Germination Resistance

Results of the three-way factorial analysis of variance for the germination resistance (GR) data are presented in Table 4.1. Significant main effects due to temperature and cultivar are indicated, and the main effect due to seed source is not significant. However, the interactions cultivar X temperature, seed source X

Table 4.1: Analysis of variance of germination resistance (GR) data (hours) for 15 six-row barley cultivars from 2 seed sources, assayed at 6 temperatures.

Source of Variation	Degrees of Freedom	Mean Square	Error Mean Square	F Ratio
Temperature (T)	5	1546500	1585	975.7 *
Replicate within T (R(T))	18	1585		
Cultivar (C)	14	9625	75	127.8 *
C*T	70	2008	75	26.7 *
C*R(T)	252	75		
Seed Source (S)	1	249	377	< 1.0 ns
S*T	5	8524	377	22.6 *
S*R(T)	18	377		
S*C	14	804	71	11.3 *
S*C*T	70	427	71	6.0 *
S*C*R(T)	252	71		
Total	719			

* = significant at P=0.05; ns = not significant.

temperature, seed source X cultivar, and seed source X cultivar X temperature are all significant. The significant cultivar effect and the non-significant effect of seed source provide the primary evidence that the observed differences in germination rate are attributable, at least in part, to genetic differences among cultivars.

The expression of plant phenotype, in this case GR measurements, is influenced by environment and by genotype. The experimental design and subsequent analysis of variance used in this experiment allows for mathematical estimation and separation of the influence imposed upon an individual experimental unit by both environmental and genotypic differences. By assaying seed samples produced in two different growing seasons, the effects on GR due to genotypic differences among cultivars can be assessed separately from the effects of environmental influences imposed on the developing seeds. However, for logistic reasons this experiment is somewhat limited in scope, and inferences made from analysis of the results must be qualified.

The main limitation arises from the testing of only two different seed sources - the minimum number to allow comparison. The seed lots used were from different years, but were both produced at the Ellerslie Research Station under similar agro-climatic conditions, soil fertility status and production management routines. Therefore, these two seed sources cannot be considered as random samples from a larger population, and variation in measured data due to seed source effect is attributed to differences between these two specific seed lots (i.e. seed source is a fixed factor). The factor cultivar is also considered fixed since each of the 15 test cultivars included in the experiments are considered homozygous at all gene loci, and are not representative of the distribution of genotypes that would be present in progeny from crosses, as in breeding nurseries. They do, however, represent a good cross-section of common western Canadian six-row barley cultivars, including malt, feed, semidwarf, and tall types. Temperature also is a fixed factor because test temperatures were

chosen at intervals throughout a specific range of practical importance. Consequently, the results from the experiments in this study cannot be used as estimates of larger population variances due to either genetic or environmental differences, but can be used in comparison to results from other similar studies and to predict the types of results expected if these experimental procedures are repeated using random samples from a larger population of interest.

Cultivar mean GR values are presented in Table 4.2, arranged in the order of ranking (from lowest to highest GR) of the 20°C seed source 1 assay (upper left column). Bracketed numbers following the means indicate ranking within each column. The statistics at the foot of each column were calculated from one-way analysis of variance of the 15 cultivars within each column. Tukey's honestly significant difference (HSD) procedure is used to test for differences among means within columns. This multiple comparisons of means test was used because it produces a single confidence interval for all possible comparisons, and it is conservative enough to maintain the probability of Type I error at the P=0.05 level among all 15 means (Sokal and Rohlf, 1980). In cases where the F ratio from one-way analysis of variance is not significant the HSD was not calculated and *ns* (no significant difference at P=0.05) is indicated. In sections to follow, the data from other assay procedures are presented in this format, maintaining the same order of cultivars in tables.

At all assay temperatures significant differences in GR among cultivars were detected (Table 4.2). Also, the combined analysis of variance (Table 4.1) indicates significant cultivar X temperature interaction. A question of importance in this type of comparison is whether any of the cultivars display crossover interaction behavior at different temperatures (Baker, 1988). For example, do any particular cultivars perform poorly at low temperature and improve in relative ranking as temperature increases, or vice versa? This question can be addressed by examination of the data in Table 4.2.

Table 4.2: Germination resistance (GR) means (hours) for 15 six-row barley cultivars from 2 seed sources, assayed at 6 temperatures. Bracketed numbers following the GR values indicate rank within temperature.

Seed Source 1 (1985)

	20 °C	15 °C	10 °C	7.5 °C	5 °C	2.5 °C
Conquest	35.0 (1)	45.8 (1)	74.3 (6)	84.9 (1)	146.1 (2)	280.8 (2)
Bonanza	35.4 (2)	46.0 (2)	71.1 (4)	88.1 (4)	147.9 (3)	274.6 (1)
Argyle	36.1 (3)	49.6 (3)	67.2 (1)	86.0 (2)	149.1 (4)	313.0 (4)
Otal	36.4 (4)	51.2 (4)	73.8 (5)	87.3 (3)	138.0 (1)	310.6 (3)
BT 522	37.1 (5)	61.3 (9)	81.4 (8)	96.9 (5)	162.0 (7)	363.1 (7)
Heartland	37.2 (6)	53.9 (5)	87.1 (11)	99.1 (8)	161.9 (6)	345.5 (6)
Klondike	37.9 (7)	54.4 (6)	68.2 (3)	97.7 (7)	170.1 (10)	334.4 (5)
BT 518	38.4 (8)	71.1 (13)	95.0 (15)	114.7 (14)	189.9 (13)	413.3 (15)
Johnston	38.5 (9)	71.5 (14)	77.4 (7)	99.9 (9)	162.2 (8)	382.3 (10)
Jackson	38.6 (10)	74.2 (15)	67.4 (2)	97.6 (6)	155.1 (5)	407.8 (13)
Leduc	38.8 (11)	61.6 (10)	84.2 (9)	106.9 (12)	192.7 (14)	391.2 (11)
Galt	39.0 (12)	61.2 (8)	94.7 (14)	104.8 (10)	188.3 (12)	368.6 (8)
Diamond	40.5 (13)	65.0 (11)	89.1 (12)	108.2 (13)	180.3 (11)	377.8 (9)
Empress	40.7 (14)	69.3 (12)	84.6 (10)	105.0 (11)	168.8 (9)	409.3 (14)
Samson	44.3 (15)	59.9 (7)	92.0 (13)	122.2 (15)	210.4 (15)	400.7 (12)
Mean	38.3	59.7	80.5	100.0	168.2	358.2
SE	0.5	1.2	2.2	2.3	5.8	10.1
HSD	2.5	6.1	11.2	11.7	29.6	51.5
CV	2.8 %	4.1 %	5.4 %	4.6 %	6.8 %	5.6 %

Seed Source 2 (1986)

	20 °C	15 °C	10 °C	7.5 °C	5 °C	2.5 °C
Conquest	31.3 (2)	45.2 (3)	73.4 (2)	87.8 (1)	176.1 (4)	255.6 (1)
Bonanza	30.8 (1)	45.2 (4)	78.6 (5)	94.0 (4)	171.4 (2)	266.4 (3)
Argyle	32.0 (4)	44.8 (2)	75.6 (4)	93.8 (3)	174.5 (3)	272.0 (4)
Otal	31.8 (3)	52.1 (7)	70.5 (1)	92.3 (2)	182.4 (6)	284.1 (5)
BT 522	32.8 (5)	44.7 (1)	75.0 (3)	100.0 (6)	168.6 (1)	264.0 (2)
Heartland	36.0 (8)	51.7 (6)	85.5 (8)	109.3 (9)	192.0 (8)	348.8 (9)
Klondike	34.3 (7)	50.1 (5)	84.2 (7)	99.3 (5)	185.1 (7)	331.4 (6)
BT 518	33.0 (6)	59.4 (11)	88.3 (9)	121.8 (12)	195.7 (11)	357.0 (12)
Johnston	38.3 (9)	58.0 (9)	91.1 (14)	108.0 (8)	193.9 (10)	338.5 (8)
Jackson	40.0 (11)	66.8 (15)	81.8 (6)	103.8 (7)	178.7 (5)	359.9 (13)
Leduc	41.0 (12)	59.2 (10)	88.7 (12)	122.8 (13)	200.9 (13)	367.9 (14)
Galt	38.8 (10)	60.2 (12)	88.4 (10)	124.5 (14)	202.8 (14)	355.5 (10)
Diamond	43.5 (14)	64.0 (14)	96.0 (15)	114.3 (11)	209.5 (15)	420.7 (15)
Empress	41.8 (13)	53.5 (8)	88.8 (13)	109.5 (10)	193.0 (9)	336.5 (7)
Samson	45.5 (15)	61.5 (13)	88.4 (11)	126.5 (15)	199.9 (12)	356.8 (11)
Mean	36.7	54.4	83.6	107.2	188.3	327.7
SE	1.5	1.2	2.4	2.2	3.2	6.9
HSD	7.5	6.1	12.2	11.2	16.3	35.2
CV	8.2 %	4.2 %	5.6 %	4.1 %	3.4 %	4.2 %

SE and CV = standard error and coefficient of variability of means in columns.
HSD = Tukey's honestly significant difference (P=0.05) among means in columns.

By applying the HSD intervals, the 15 cultivars in each column can generally be divided into three groups of five cultivars per group. The first group contains the five cultivars with lowest GR, ranked 1 to 5 in the table. The middle group contains the cultivars ranked 6 to 10, and the last group contains the cultivars ranked 11 to 15. Statistically, all cultivars in the first group are different from all cultivars in the last group. Some of the cultivars in the middle group are significantly different from some of the cultivars in the first group, and some in the middle group are different from some in the last group. In general, any two cultivars must be five or more ranking positions apart to be significantly different. Thus, the cultivars within each of the three groups are not significantly different from one another. These generalizations do not hold absolutely true for all the data columns in Table 4.2, but provide a close and workable approximation for identifying the cultivars that contribute most to the temperature X cultivar interaction. In practice the genotypes with extreme values in populations are usually of most interest to plant breeders.

Cultivars that rank in the first group at one temperature and in the last group at another temperature can be identified from Table 4.2, but this occurs in only three cases: Heartland from seed source 1, and Jackson from both of seed source 1 and 2. Heartland falls into this category because of rank assignments of 11 in the 10°C assay and 5 in the 15°C assay of seed source 1. All other assays rank Heartland in the middle class. Jackson's response to differing assay temperatures appears much more erratic, ranking in the first group at 5 and 10°C and in the last group at 2.5, 15, and 20°C. No meaningful trend in ranking change over different temperatures can be identified, but it is worth noting that Jackson's variability is very similar for both seed sources.

Several other cultivars change in ranking over assay temperatures from one group to the next adjacent group (e.g. the first group to the middle group). For example, Samson ranks 7th in the 15°C seed source 1 assay but in all other assays ranks consistently in the last group. BT 518 ranks in the middle group at 20°C and in the last

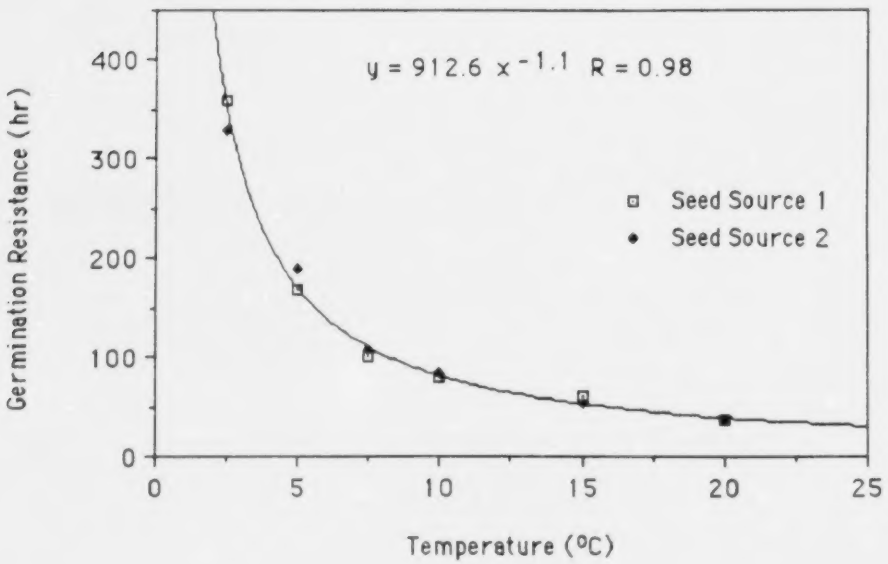
group at nearly all other assay temperatures. But again, no useful trends in cultivar X temperature crossover interactions are obvious. The apparently random rank changes that are evident in Table 4.2 are likely due to a combination of natural variability of seeds within the seed lots, and variability due to experimental error.

Cultivars that consistently rank in the first group (low GR) include the malting types Conquest, Bonanza, and Argyle, and the early maturing feed type Otal. At the other end of the distribution the cultivars Leduc, Diamond, and Samson rank, with a few exceptions, into the last group (high GR). Overall, the results in Table 4.2 indicate that GR differs genetically among these 15 cultivars, and that the differences tend to be expressed with relative consistency across the spectrum of assay temperatures. The next question to be answered is what assay temperature would be most appropriate for screening large numbers of genotypes for differences in GR?

In Figure 4.1 the GR means of all 15 test cultivars from each seed source and assay temperature (means of columns in Table 4.2) are averaged together and plotted against temperature. The resulting curve depicts an overall decline in GR between 2.5 and 20°C. Between 2.5 and 5°C the decline in GR with increasing temperature is relatively fast, as indicated by the sharply sloping line segment. From 5 to 10°C the slope of the line decreases substantially, and above 10°C the line slope remains constant and relatively shallow. Below the 5 to 10°C range a small change in temperature has a much greater effect on GR than does a small temperature change in the 10 to 20°C range.

This changing effect of temperature on GR can be illustrated numerically by calculating temperature quotient (Q_{10}) ratios over the range of assay temperatures. Q_{10} ratios can be used to compare the rates of biochemical or growth processes at

Figure 4.1: Germination resistance (GR) means of 2 seed sources at 6 assay temperatures.



different temperatures according to the following formula from Salisbury and Ross (1978):

$$Q_{10} = (k_2/k_1)^{10/T_2-T_1}$$

where, T_1= lower temperature, T_2= higher temperature,

k_1= rate at lower temperature, k_2= rate at higher temperature.

The GR values can be substituted into the Q_{10} equation for k since they are actually the mean times to germination of 100 seeds, or the average rate of germination measured in hours per germination event. In Table 4.3 Q_{10} values are shown for the 15 test cultivars at each temperature interval using the means of seed source 1 and 2 averaged together.

The Q_{10} values display a similar trend for all cultivars. The largest values occur at the 2.5 to 5°C and 5 to 7.5°C intervals. At the higher temperature intervals, 7.5 to 10°C, 10 to 15°C, and 15 to 20°C, the Q_{10} values stabilize in the 1.6 to 3.6 range. Jordan et. al. (1985) found a similar trend in Q_{10} values for rootlet growth rate data from wheat seedlings 5 days after imbibition. In their study Q_{10} values ranged from 4.3 to 10.5 for intervals between 5 and 12°C, and from 1.0 to 1.7 for intervals between 12 and 25°C. The authors postulate that a large Q_{10} change in the 10 to 12°C range may be indicative of a critical transition temperature.

In this experiment the critical temperature for change in Q_{10} values appears to be near 7.5°C. Considering the hypothesis that cellular membranes undergo changes in physical state at chilling temperatures (Lyons and Raison, 1970), and that these changes cause disruption of normal cellular biochemical activities and growth processes (Caldwell and Whitman, 1987), it seems reasonable to conclude that germination of the 15 test cultivars in this study is impeded at temperatures below 7.5°C by properties other than the normal thermal dynamics of biochemical reaction rates. Evidence has been reported indicating the existence of within species genetic

Table 4.3: Temperature quotient (Q_{10}) ratios for germination resistance (GR) means of 15 cultivars calculated at intervals between 20 and 2.5°C.

	20-15°C	15-10°C	10-7.5°C	7.5-5°C	5-2.5°C
Conquest	1.9	2.6	1.9	12.1	7.7
Bonanza	1.9	2.7	2.2	9.5	8.2
Argyle	1.9	2.3	2.5	10.5	10.7
Otal	2.3	2.0	2.4	10.1	11.9
BT 522	2.3	2.2	2.5	7.9	12.9
Heartland	2.1	2.7	2.1	8.3	14.8
Klondike	2.1	2.1	2.8	10.6	12.3
BT 518	3.3	2.0	2.8	7.1	15.9
Johnston	2.8	1.7	2.3	8.6	16.8
Jackson	3.2	1.6	3.3	7.5	28.0
Leduc	2.3	2.0	3.1	8.6	13.8
Galt	2.4	2.3	2.5	8.5	11.8
Diamond	2.4	2.1	2.1	9.4	17.6
Empress	2.2	2.0	2.3	8.1	18.1
Samson	1.8	2.2	3.6	7.4	11.6
Mean	2.3	2.1	2.5	8.9	13.8

variability in membrane composition properties that impart resistance to phase change at chilling temperatures (Markhart, 1986; Wang, 1982). It appears that the cultivars assayed in the present study are genetically variable in GR, but are essentially homogeneous with respect to critical phase change temperature. However, this evidence does not preclude the possibility that samples from larger, more genetically diverse populations could contain genotypes with significantly superior, or inferior, tolerance to chilling temperatures. Germination rate assays conducted at temperatures near or below 7.5°C would be most likely to detect these genotypes.

4.1.2 Seedling Total Dry Weight

Analysis of variance results from the seedling total dry weight (TDW) assays are shown in Table 4.4. Significant F ratios are indicated for temperature and cultivar main effects, and for the temperature X cultivar interaction. All other terms in the analysis are not significant. As with the GR assays, the detection of cultivar differences in seedling TDW and the lack of significant seed source effect indicates that genetic variability in growth rate exists among the 15 cultivars . However, the variability in TDW among cultivars appears to be less extensive than the variability in GR. This is demonstrated by using the HSD intervals to compare cultivars within columns in Table 4.5. For most assay temperatures, means within columns are not significantly different unless they are ranked eight or more positions apart. In general the two top ranked cultivars (rank numbers 1 and 2) are significantly different from the two lowest ranked cultivars (rank numbers 14 and 15).

The low number of significantly different cultivar mean comparisons is due, at least in part, to relatively large experimental error variances, as indicated by large CV values (Table 4.5). CV values for GR averaged near 5.0% (Table 4.2) while CV values for TDW averaged 14.5% . The methodology used here for TDW assays is prone to error at numerous steps throughout the procedure. Uncontrollable temperature and light

Table 4.4: Analysis of variance of seedling total dry weight (TDW) data (g/10 plants) for 15 six-row barley cultivars from 2 seed sources, assayed at 5 temperatures.

Source of Variation	Degrees of Freedom	Mean Square	Error Mean Square	F Ratio
Temperature (T)	4	17.76	5.25	3.4 *
Rep in Temp R(T)	15	5.25		
Cultivar (C)	14	6.54	0.23	28.4 *
C*T	56	0.50	0.23	2.2 *
C*R(T)	210	0.23		
Seed Source (S)	1	3.35	0.96	3.5 ns
S*T	4	2.89	0.96	3.0 ns
S*R(T)	15	0.96		
S*C	14	0.39	0.26	1.5 ns
S*C*T	56	0.27	0.26	1.0 ns
S*C*R(T)	210	0.26		
Total	599			

* = significant at P=0.05; ns = not significant.

Table 4.5: Seedling total dry weight (TDW) means (g/10 plants) for 15 six-row barley cultivars, from 2 seed sources, assayed at 5 temperatures. Bracketed numbers following TDW values indicate ranking within temperature.

Seed Source 1 (1985)

	20 °C	15 °C	10 °C	7.5 °C	5 °C
Conquest	4.04 (4)	2.86 (8)	3.43 (6)	4.03 (4)	2.87 (6)
Bonanza	3.52 (8)	2.75 (11)	3.40 (7)	3.62 (6)	3.21 (3)
Argyle	3.23 (10)	2.75 (10)	3.16 (10)	2.56 (15)	2.37 (14)
Otal	3.11 (13)	2.59 (13)	2.76 (15)	2.72 (14)	2.56 (11)
BT 522	4.16 (2)	2.97 (7)	4.15 (1)	3.48 (7)	3.75 (1)
Heartland	3.79 (6)	3.15 (3)	3.27 (8)	3.70 (5)	2.63 (9)
Klondike	3.20 (12)	2.56 (14)	2.89 (14)	2.74 (13)	2.58 (10)
BT 518	3.28 (9)	2.44 (15)	3.01 (11)	3.03 (11)	2.42 (13)
Johnston	4.10 (3)	3.74 (1)	4.09 (2)	5.09 (1)	3.24 (2)
Jackson	4.40 (1)	3.55 (2)	3.52 (4)	4.12 (3)	2.87 (5)
Leduc	3.20 (11)	3.01 (5)	3.49 (5)	3.13 (9)	2.66 (8)
Galt	2.94 (14)	2.66 (12)	2.99 (12)	3.24 (8)	2.13 (15)
Diamond	3.99 (5)	3.01 (4)	3.26 (9)	3.06 (10)	2.76 (7)
Empress	3.57 (7)	3.00 (6)	3.66 (3)	4.90 (2)	3.04 (4)
Samson	2.43 (15)	2.79 (9)	2.94 (13)	2.91 (12)	2.54 (12)
Mean	3.53	2.92	3.33	3.49	2.77
SE	0.21	0.17	0.14	0.39	0.26
HSD	1.07	0.87	0.71	1.99	1.33
CV	11.8 %	11.9 %	8.5 %	22.5 %	18.5 %

Seed Source 2 (1986)

	20 °C	15 °C	10 °C	7.5 °C	5 °C
Conquest	3.69 (6)	3.57 (10)	3.43 (7)	3.61 (10)	2.81 (4)
Bonanza	3.67 (7)	3.62 (9)	3.11 (11)	4.42 (4)	2.73 (5)
Argyle	3.22 (12)	3.72 (5)	3.18 (9)	2.90 (15)	2.39 (11)
Otal	3.45 (11)	3.66 (8)	2.86 (13)	3.75 (7)	2.44 (10)
BT 522	3.90 (4)	3.71 (6)	3.67 (3)	4.65 (2)	3.21 (2)
Heartland	3.59 (9)	3.83 (3)	3.47 (6)	3.71 (8)	2.48 (9)
Klondike	3.82 (5)	3.01 (14)	3.54 (5)	4.21 (6)	2.64 (6)
BT 518	2.92 (13)	3.26 (12)	2.72 (15)	3.21 (14)	1.97 (15)
Johnston	4.02 (1)	3.94 (1)	4.78 (1)	5.04 (1)	3.41 (1)
Jackson	3.98 (3)	3.81 (4)	3.21 (8)	4.56 (3)	2.61 (8)
Leduc	3.65 (8)	3.33 (11)	3.16 (10)	3.33 (13)	2.29 (12)
Galt	2.64 (15)	3.06 (13)	2.97 (12)	3.48 (11)	2.19 (13)
Diamond	4.01 (2)	3.69 (7)	3.60 (4)	3.68 (9)	2.61 (7)
Empress	3.49 (10)	3.85 (2)	4.27 (2)	4.31 (5)	3.06 (3)
Samson	2.77 (14)	2.51 (15)	2.85 (14)	3.41 (12)	2.14 (14)
Mean	3.52	3.50	3.39	3.88	2.60
SE	0.25	0.21	0.22	0.30	0.24
HSD	1.28	1.07	1.12	1.53	1.22
CV	14.4 %	12.1 %	12.6 %	15.5 %	18.4 %

SE and CV = standard error and coefficient of variability of means in columns.
HSD = Tukey's honestly significant difference (P=0.05) among means in columns.

gradients in growth cabinets are only partially compensated by blocking of replications and periodic re-randomization of pots. Determining the proper time to harvest plants involved estimating leaf stage of the majority of plants, a judgement decision that could have inadvertently biased some cultivars over others. The washing of soil from roots before weighing plants also presented opportunity for error since it is impossible to remove all the soil and not lose some root tissue.

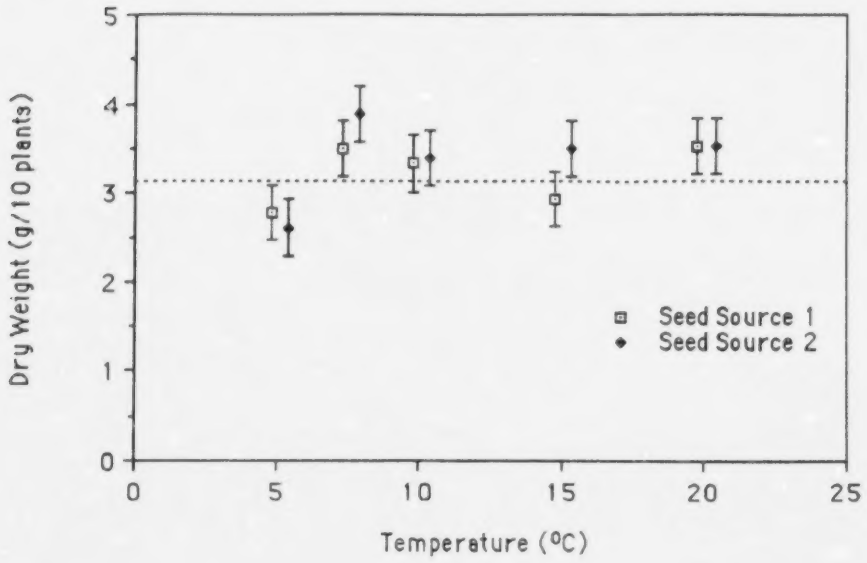
In spite of the large experimental error, some significant TDW differences were detected among cultivars with reasonable consistency across the range of assay temperatures and seed sources. Johnston, Jackson and BT 522 usually ranked high, with Johnston ranking highest in TDW in the majority of the assays. Samson, Galt and BT 518 ranked lowest in TDW most frequently. At all temperatures Johnston was significantly superior in TDW to Samson. Note that of the faster germinating cultivars (Table 4.2), Conquest and Bonanza usually rank in the high to mid TDW range, while Argyle and Otal rank in the mid to low TDW range. De La Soujeole (1985) reported similar independence of genotypic differences in germination rate and seedling growth rate among a number of sorghum lines tested for low temperature vigor. It is likely that the critical physiological processes limiting seedling growth rate are different from those limiting germination rate, and these different processes are probably controlled by different gene complexes.

The significant cultivar X temperature interaction (Table 4.4) is caused by the behavior of cultivars like Argyle and Klondike. Argyle tends to rank low in TDW except for the 15°C seed source 2 assay where it ranks fifth. Klondike ranked relatively low in TDW at all temperatures in seed source 1 assays, but ranked high in all seed source 2 assays except 15°C. As with the GR results, no cases are evident where a cultivar shows consistent improvement in relative performance as assay temperature increases or decreases.

The overall effect of varying temperature on TDW is displayed graphically in Figure 4.2. The combined TDW means of all 15 cultivars are plotted for each seed source and assay temperature. Error bars indicate least significant difference intervals (LSD) for comparisons among temperature means (Sokal and Rohlf, 1980). TDW does not progressively increase with temperature, as did germination rate (Figure 4.1), because seedling leaf stage was used as the criterion for determining the time duration of each assay. Assays at low temperatures took much longer to complete than did assays at higher temperatures, but plants from all assays were harvested at the same developmental stage.

Lowest mean TDW occurs at 5°C for both seed sources. For seed source 2 the 5°C mean TDW is significantly lower than all other temperature means. With seed source 1 only the 7.5 and 20°C means are significantly greater than 5°C means. Between 7.5°C and 20°C TDW assay temperature means do not differ significantly for either seed source. The significant reduction in TDW of seedlings at 5°C may be an indication that the critical temperature at which membrane phase transition occurs in developing seedlings of these 15 cultivars lies between 7.5 and 5°C, slightly lower than the critical temperature during germination. A number of plant species have been reported to be more susceptible to chilling injury during germination than during vegetative growth (Lyons, 1973; Maguire, 1972). Low temperature damage to mitochondria or thylakoid membranes, as reported from previous research with barley (Lyons and Raison, 1970; Smillie et. al., 1978; Smillie and Hetherington, 1983), could impede the normal metabolic and growth processes of seedlings resulting in stunted growth even though ontogenic development proceedes.

Figure 4.2: Seedling total dry weight (TDW) means (g/10 plants) of 2 seed sources at 5 assay temperatures. Error bars indicate least significant difference (LSD) (P=0.05) for comparison of means among temperatures. The horizontal dotted line is included to aid in making visual comparisons.



4.2: Experiment 2. Field Trials

Field trials were conducted during the summers of 1987 and 1988 at two locations each year, Edmonton and Ellerslie. Seed source 1, produced in 1985, was used to establish the 1987 plots and seed source 2, produced in 1986, was used for 1988 plots. The two seed sources were utilized in different years of the field trials because there was not sufficient quantity of either seed source to permit the testing of a common seed lot over all four site-years. By delaying the planting of seed source 2 by one year the two seed lots were essentially the same age at seeding time (i.e approximately 20 months from harvest to planting). Gordon (1972) has shown that the germination rate of barley and wheat cultivars increases with time from harvest due to gradual loss of dormancy, and after a period of time in storage germination rate begins to decline due to ageing. Utilizing the two seed sources in successive years allowed for testing of the 15 cultivars at two different field locations each year, in plots established from seed lots common in age.

At each field site plots were established in a split-plot design with seeding dates as main plots and cultivars as sub-plots. Seeding dates were chosen to provide a range in soil temperature at seeding time (Table 4.6). The first seeding date treatments were established at each site as early as field conditions permitted. The second and third seeding dates were established at either two or three week intervals following the first date, depending upon weather conditions and change in soil temperature. During both growing seasons of the study early spring weather conditions were uncommonly warm (Table 4.7). Average air temperatures were twice the normal for the general area during April, and growing degree days were well above normal during April and May of both years. As a result, the first seeding dates were very early in relation to usual commercial practice in central Alberta; however, the intended range of seedbed temperatures was achieved (Table 4.6). Daily and weekly mean soil

Table 4.6: Daily and weekly mean soil temperatures (°C) from 1987 and 1988 field plots measured at seeding depth (4 cm). Daily means are an average of two readings taken at 1200 and 2400 hours on the day of seeding, and weekly means are an average of the daily mean temperatures for the 7 days after seeding.

Site (Year)	Edmonton (1987)			Ellerslie (1987)		
Seeding Date	April 22	May 6	May 27	April 29	May 13	June 3
Daily Mean	4	14	18	9	10	17
Weekly Mean	6	13	16	10	12	17

Site (Year)	Edmonton (1988)			Ellerslie (1988)		
Seeding Date	April 14	April 29	May 11	April 21	May 4	May 19
Daily Mean	4	9	13	3	6	14
Weekly Mean	3	7	14	5	9	16

Table 4.7: Monthly average air temperature, growing degree days (base 5°C) and precipitation measured in the general area of the field plots. Source: Environment Canada Meteorological Station, Edmonton International Airport. Thirty-year averages include the years 1951 to 1980.

1987	April	May	June	July	August
Air Temperature (°C)	7.0	11.2	15.5	15.8	12.6
30-year average	3.2	10.1	14.1	15.8	14.8
Growing Degree Days	76	203	317	335	273
30-year average	38	164	273	336	304
Precipitation (cm)	1.1	8.0	6.8	12.0	9.6
30-year average	2.0	4.2	7.7	9.2	7.8

1988	April	May	June	July	August
Air Temperature (°C)	6.0	12.5	15.8	15.8	15.1
30-year average	3.2	10.1	14.1	15.8	14.8
Growing Degree Days	56	233	323	334	312
30-year average	38	164	273	336	304
Precipitation (cm)	2.2	1.6	12.1	13.3	10.3
30-year average	2.0	4.2	7.7	9.2	7.8

temperatures at seeding depth ranged from near 5°C for the first seeding dates to 14°C and above for the third seeding dates during both years. Soil moisture at seeding depth was adequate for germination at all seeding dates in 1987 . In 1988 the combination of above average air temperature and below average precipitation in May resulted in relatively dry seedbed conditions for the second and third seeding dates at both locations.

4.2.1 Seedling Emergence Rate

Seedling emergence rate data, recorded as days to 75% emergence (DE), were subjected to analysis of variance at three levels: a combined analysis of all four site-years (Table 4.8); individual analysis of each site-year (Table 4.9); and finally, one-way analysis of each seeding date treatment containing all 15 test cultivars (Table 4.10). The need to examine all three levels of analysis is explained in a discussion of series field experiments by Steel and Torrie (1980). In cultivar trials, the combined analysis provides information concerning the consistency of cultivar performance from year to year and site to site. If cultivar differences are significant but the interaction cultivar X site-year is not significant it can be concluded that the best performing cultivars encompass a general superiority for the characteristic under test, within the range of environments in the study. However, error variances tend to differ considerably among site-years, and consequently the use of pooled error terms can distort the F tests for treatment comparisons. Individual site-year analysis provides a more accurate assessment of main treatment effects and within site-year interactions. In this experiment, one-way analysis of variance for each seeding date treatment is used to derive standard errors for cultivar mean comparisons because of error variance heterogeneity due to soil temperature differences.

The combined analysis of DE data (Table 4.8) indicates significant site-year, seeding date and cultivar main effects as well as a significant seeding date X site-year

Table 4.8: Combined site-year analysis of variance of days to 75% emergence (DE) data from 4 site-years for 15 six-row barley cultivars at 3 seeding dates.

Source of Variation	Degrees of Freedom	Mean Square	Error Mean Square	F Ratio
Site-Year (SY)	3	1475.5	2.1	702.6 *
Replicate within SY (R(SY))	12	2.1		
Seeding Date (SD)	2	1983.0	3.6	550.8 *
SD x SY	6	101.0	3.6	28.1 *
SD x R(SY)	24	3.6		
Cultivar (C)	14	26.3	2.0	13.2 *
C x SY	42	2.2	2.0	1.1 ns
C x SD	28	2.0	2.0	1.0 ns
C x SY x SD	84	1.6	2.0	<1.0 ns
residual	504	2.0		
total	719			

* = significant at P=0.05; ns = not significant.

Table 4.9: Individual site-year analysis of variance of days to 75% emergence (DE) data for 15 six-row barley cultivars at 3 seeding dates from the Edmonton and Ellerslie field plots.

Source of Variation	Degrees of Freedom	Mean Square	Error Mean Square	F Ratio
		Edmonton (1987)		
Replication (R)	3	5.19		
Seeding Date (SD)	2	457.00	2.25	203.1 *
R x SD	6	2.25		
Cultivar (C)	14	4.90	0.64	7.7 *
C x SD	28	0.38	0.64	<1.0 ns
residual	126	0.64		
total	179			
		Ellerslie (1987)		
Replication (R)	3	2.03		
Seeding Date (SD)	2	183.52	1.64	111.9 *
R x SD	6	1.64		
Cultivar (C)	14	10.78	0.29	37.2 *
C x SD	28	1.37	0.29	4.7 *
residual	126	0.29		
total	179			
		Edmonton (1988)		
Replication (R)	3	0.15		
Seeding Date (SD)	2	430.00	7.93	54.2 *
R x SD	6	7.93		
Cultivar (C)	14	9.34	3.78	2.5 *
C x SD	28	2.65	3.78	<1.0 ns
residual	126	3.78		
total	179			
		Ellerslie (1988)		
Replication (R)	3	1.14		
Seeding Date (SD)	2	1215.00	2.47	491.9 *
R x SD	6	2.47		
Cultivar (C)	14	10.74	2.23	4.8 *
C x SD	28	2.50	2.23	1.1 ns
residual	126	2.23		
total	179			

* = significant at P=0.05; ns = not significant.

Table 4.10: Mean days to 75% emergence (DE) for 15 six-row barley cultivars grown at 2 central Alberta locations at different seeding dates. Means within columns followed by the same letter are not significantly different (P=0.05).

	Edmonton (1987)			Ellerslie (1987)		
Seeding Date	April 22	May 6	May 27	April 29	May 13	June 3
Conquest	12.5 ab	8.2 ab	7.5 ab	11.0 a	10.0 ab	7.5 a
Bonanza	12.0 a	8.2 ab	7.5 ab	10.2 a	9.5 a	7.5 a
Argyle	12.0 a	8.2 ab	7.2 a	10.2 a	10.0 ab	7.8 ab
Otal	12.5 ab	8.0 a	7.0 a	10.2 a	9.8 ab	7.8 ab
BT 522	13.0 ab	8.8 ab	7.8 abc	10.5 a	9.2 a	7.5 a
Heartland	13.0 ab	8.2 ab	7.5 ab	11.8 ab	10.0 ab	8.2 abc
Klondike	12.5 ab	8.2 ab	7.8 abc	10.5 a	9.8 ab	8.0 abc
BT 518	14.0 bc	9.0 ab	8.5 bc	13.0 bc	11.0 bc	9.2 cd
Johnston	13.0 ab	9.0 ab	8.2 abc	10.5 a	10.0 ab	7.5 a
Jackson	12.5 ab	8.8 ab	7.5 ab	13.2 bc	9.8 ab	8.0 abc
Leduc	13.5 abc	9.0 ab	8.8 c	13.0 bc	10.5 ab	8.5 abc
Galt	13.0 ab	8.8 ab	8.0 abc	13.8 c	11.2 c	8.8 bc
Diamond	14.0 bc	9.5 bc	8.5 c	12.8 bc	11.0 bc	9.2 cd
Empress	13.5 abc	9.0 ab	8.0 abc	10.5 a	10.0 ab	7.8 a
Samson	15.0 c	10.5 c	9.0 c	14.5 c	12.0 c	10.2 d
Mean	13.1	8.8	7.9	11.7	10.2	8.2
SE	0.38	0.28	0.25	0.36	0.27	0.25
HSD	1.9	1.4	1.3	1.8	1.4	1.3
CV	5.8%	6.2%	6.4%	5.5%	5.2%	6.1%

	Edmonton (1988)			Ellerslie (1988)		
Seeding Date	April 14	April 29	May 11	April 21	May 4	May 19
Conquest	17.8 abcd	16.5	12.5	16.0 a	13.5	8.8
Bonanza	18.2 abcd	15.2	12.5	16.5 ab	13.5	9.5
Argyle	16.8 ab	15.2	12.5	16.0 a	13.5	9.0
Otal	16.5 a	16.5	12.5	18.0 ab	13.5	8.0
BT 522	18.0 abcd	15.2	11.2	17.5 ab	12.8	9.2
Heartland	17.2 abc	16.5	13.8	19.0 ab	13.5	9.8
Klondike	18.2 abcd	16.5	12.5	19.0 ab	13.5	8.2
BT 518	18.0 abcd	14.0	13.8	19.0 ab	15.5	10.2
Johnston	17.5 abcd	16.5	12.5	20.0 ab	14.2	10.0
Jackson	18.8 bcde	14.0	11.2	18.0 ab	14.2	9.8
Leduc	19.5 de	16.5	13.8	19.0 ab	14.2	10.2
Galt	18.0 abcd	16.5	13.8	19.0 ab	14.2	8.2
Diamond	19.0 cde	16.5	13.8	21.0 b	14.2	10.5
Empress	18.5 abcde	15.2	12.5	18.0 ab	13.5	9.5
Samson	20.5 e	17.8	13.8	21.0 b	16.0	11.0
Mean	18.2	15.9	12.8	18.5	14.0	9.5
SE	0.42	1.28	1.33	0.92	1.08	0.67
HSD	2.1	ns	ns	4.7	ns	ns
CV	4.6%	16.1%	20.7%	10.0%	15.1%	14.1%

SE and CV = standard error and coefficient of variability of means in columns.
HSD = Tukey's honestly significant difference (P=0.05) among means in columns (ns = no significant difference among means in columns).

interaction. The cultivar X site-year interaction is not significant, indicating that the observed cultivar differences in emergence rate are reproducible and to some extent genetically determined. In performing the combined analysis two primary assumptions were made. First, it is assumed that seed sources 1 and 2 can be considered equivalent samples of the test cultivars. This appears to be a reasonable generalization since the seed source main effects for both GR and TDW in Experiment 1 were not significant. Second, it is assumed that seeding date treatments at all site-years have enough similarity so that meaningful comparisons can be made. For example, are first seeding date conditions similar among site-years and different from the second and third seeding dates? Since the primary environmental condition under test is seedbed soil temperature, the temperature data in Table 4.6 provide validity of this assumption. With the exception of the 1987 Ellerslie site, soil temperatures increase consistently with later seeding dates to provide the desired range of test temperatures. Other uncontrollable environmental conditions, such as precipitation and air temperature (Table 4.7), vary among seeding dates and years, and their effects are reflected in the one-way analysis of variance and mean comparisons of Table 4.9. Consistency of seeding date conditions among site-years is obviously imperfect, as indicated by the significant seeding date X site-year interaction, but the field trials do provide reasonable comparisons for evaluation of growth chamber assays.

Individual site analysis of variance (Table 4.9) shows significant seeding date and cultivar differences for all site-years. A significant cultivar X seeding date interaction is indicated only for the 1987 Ellerslie site. At this site the narrowest range in soil temperatures among seeding dates occurs (Table 4.6), and the error mean square for testing the cultivar X seeding date interaction is small compared to the other sites (Table 4.9), resulting in a significant F test for the interaction term in the analysis of variance. Table 4.10 contains the DE means from the four field trials

Cultivars are arranged in the same order as in Experiment 1 data tables, and significant differences among cultivars are indicated by the letters following each DE mean value. The letter assignments are based on the HSD values derived from one-way analysis of variance. Means within columns with a common letter are not significantly different (P=0.05). This format is used for field trial results, rather than rank assignments, because of the frequent occurrence of identical means.

Cultivar differences in DE were significant at all seeding dates in 1987, but in 1988 differences were detected only in the earliest seeding date treatments at both sites (Table 4.10). In 1988 unusually warm air temperatures and below normal precipitation in late April and May resulted in relatively dry seedbed conditions for the second and third seeding dates. Random variability in seedbed moisture throughout the plots caused 'patchy' germination, irrespective of cultivar treatments, which considerably increased error variances. Note in Table 4.10 the large CV values for the later seeding dates in 1988.

Similar to the GR results, certain cultivars consistently rank in the fastest emerging group, while other cultivars are consistently slower to emerge. Conquest, Bonanza, Argyle, and Otal are nearly always significantly lower in DE when compared to Samson and Diamond, two of the slowest emerging cultivars over all seeding dates. Leduc and BT 518, which usually rank among the slower germinating cultivars in GR assays, tend to rank in the mid-range for DE. In general, cultivar differences in field DE reflect differences in GR measured in growth cabinet assays, and display the same tendancy to be expressed with consistency at all temperatures. Results of correlation analysis between GR assays and field DE are presented in a later section.

4.2.2 Shoot Dry Weight

To assess cultivar differences in dry matter accumulation in the field the shoots from 10 seedlings per treatment were harvested at 3 and 5 weeks after seeding, oven dried and weighed. Shoot dry weight (ShDW) sampling was done twice after emergence to determine if the advantage gained by earlier emerging cultivars is maintained as the seedlings develop, or if the later emerging cultivars compensate for the delayed start with faster seedling growth. At the 3 week sampling time most plants from the earlier seeding date treatments, where soil temperatures were low, were still in the 1 to 2 leaf stage and cultivar differences in development were visually obvious in some cases. At 5 weeks cultivar differences in shoot growth could no longer be determined visually.

ShDW data was subjected to analysis of variance at three levels as was done with DE data. The combined site-year analysis results for 3 week and 5 week post seeding samples are presented in Tables 4.11a and 4.11b respectively. At both sampling times site-year, seeding date and cultivar main effects are significant. However, unlike the DE analysis, ShDW cultivar X site-year interactions are significant. A possible explanation for this interaction is that ShDW is influenced by a number of climatic conditions while DE is influenced primarily by soil temperature, provided sufficient moisture is present for germination. As can be seen from Tables 4.6 and 4.7, soil temperatures are relatively consistent among site-years but other conditions, such as growing degree days and precipitation, are variable from year to year and would likely have more effect on seedling growth than on germination and emergence. With the wide range of cultivars included in these trials there very likely are some genotypic differences in characteristics such as water use efficiency or net assimilation rate, and these differences could be responsible for significant cultivar interactions with site-year or seeding date.

Individual site analysis of variance for 3 week and 5 week ShDW sampling data (Tables 4.12a and 4.12b respectively) are consistent between the two sample dates. At all sites seeding date and cultivar effects are significant. The seeding date X cultivar interaction is significant for both 3 week and 5 week data from the 1987 Edmonton site only. Non-significance of seeding date X cultivar interaction effects may be a result of the high error variances, as indicated by the high CV values in Tables 4.13a and 4.13b. The large experimental errors, possible due to patchy soil moisture conditions within plot sites, also seem to have masked cultivar differences in ShDW in 1988 field trials, as with the DE data.

From the data in Tables 4.13a and 4.13b it can be seen that cultivars with high ShDW are not necessarily superior in germination and emergence rate . For example, Bonanza and Argyle, both fast germinating cultivars, are very often not significantly different in ShDW from Samson; and when they are different it is in an early seeding date plot (i.e. Edmonton, 1987, date 1). Conversely, Jackson frequently ranks high in ShDW and is nearly always significantly different from the lowest ranking cultivars. Jackson also ranks high in growth cabinet TDW assays in Experiment 1 but does not stand out as either high or low in GR assays. Samson consistently ranks low in both germination rate and seedling growth rate, whether assayed in growth cabinet experiments or field trials. In early seeding date plots soil and air temperatures are lower resulting in slower plant growth, hence the advantage gained by earlier emerging cultivars is still evident at the 3 and 5 week sample times. In later seeding date plots, with higher average temperatures, growth is faster and the cultivar differences in emergence are compensated for to a greater degree by differences in seedling growth rate.

A comparison of cultivar ranking between Table 4.13a and Table 4.13b reveals very similar trends at both sampling times. Thus, differences among cultivars in early seedling growth appear to be maintained as seedling development proceeds.

Table 4.11a: Combined site-year analysis of variance of shoot dry weight (ShDW) data (g/10 plants), sampled 3 weeks after seeding, from 4 site-years for 15 six-row barley cultivars at 3 seeding dates.

Source of Variation	Degrees of Freedom	Mean Square	Error Mean Square	F Ratio
Site-Year (SY)	3	43.74	0.82	53.3 •
Replicate within SY (R(SY))	12	0.82		
Seeding Date (SD)	2	452.79	0.77	588.0 •
SD x SY	6	12.43	0.77	16.1 •
SD x R(SY)	24	0.77		
Cultivar (C)	14	0.97	0.10	9.7 •
C x SY	42	0.16	0.10	1.6 •
C x SD	28	0.21	0.10	2.1 •
C x SY x SD	84	0.11	0.10	1.1 ns
residual	504	0.10		
total	719			

• = significant at P=0.05; ns = not significant.

Table 4.11b: Combined site-year analysis of variance of shoot dry weight (ShDW) data (g/10 plants), sampled 5 weeks after seeding, from 4 site-years for 15 six-row barley cultivars at 3 seeding dates.

Source of Variation	Degrees of Freedom	Mean Square	Error Mean Square	F Ratio
Site-Year (SY)	3	1962.9	107.6	18.2 •
Replicate within SY (R(SY))	12	107.6		
Seeding Date (SD)	2	12391.1	126.1	98.3 •
SD x SY	6	31.9	126.1	<1.0 ns
SD x R(SY)	24	126.1		
Cultivar (C)	14	78.5	7.8	10.1 •
C x SY	42	14.5	7.8	1.9 •
C x SD	28	14.1	7.8	1.8 •
C x SY x SD	84	7.0	7.8	<1.0 ns
residual	504	7.8		
total	719			

• = significant at P=0.05; ns = not significant.

Table 4.12a: Individual site-year analysis of variance of shoot dry weight (ShDW) data (g/10 plants), sampled 3 weeks after seeding, for 15 six-row barley cultivars at 3 seeding dates from the Edmonton and Ellerslie field plots.

Source of Variation	Degrees of Freedom	Mean Square	Error Mean Square	F Ratio
		Edmonton (1987)		
Replication (R)	3	0.37		
Seeding Date (SD)	2	167.00	0.77	216.9 *
R x SD	6	0.77		
Cultivar (C)	14	0.61	0.16	3.8 *
C x SD	28	0.31	0.16	1.9 *
residual	126	0.16		
total	179			
		Ellerslie (1987)		
Replication (R)	3	0.23		
Seeding Date (SD)	2	203.00	0.27	751.9 *
R x SD	6	0.27		
Cultivar (C)	14	0.53	0.10	5.3 *
C x SD	28	0.12	0.10	1.2 ns
residual	126	0.10		
total	179			
		Edmonton (1988)		
Replication (R)	3	0.35		
Seeding Date (SD)	2	51.00	0.47	108.5 *
R x SD	6	0.47		
Cultivar (C)	14	0.06	0.02	3.0 *
C x SD	28	0.03	0.02	1.5 ns
residual	126	0.02		
total	179			
		Ellerslie (1988)		
Replication (R)	3	2.33		
Seeding Date (SD)	2	68.36	1.58	43.3 *
R x SD	6	1.58		
Cultivar (C)	14	0.23	0.07	3.3 *
C x SD	28	0.09	0.07	1.3 ns
residual	126	0.07		
total	179			

* = significant at P=0.05; ns = not significant.

Table 4.12b: Individual site-year analysis of variance of shoot dry weight (ShDW) data (g/10 plants), sampled 5 weeks after seeding, for 15 six-row barley cultivars at 3 seeding dates from the Edmonton and Ellerslie field plots.

Source of Variation	Degrees of Freedom	Mean Square	Error Mean Square	F Ratio
		Edmonton (1987)		
Replication (R)	3	47.0		
Seeding Date (SD)	2	3163.4	67.7	46.7 *
R x SD	6	67.7		
Cultivar (C)	14	34.1	4.7	7.3 *
C x SD	28	11.1	4.7	2.4 *
residual	126	4.7		
total	179			
		Ellerslie (1987)		
Replication (R)	3	255.6		
Seeding Date (SD)	2	2935.1	259.1	11.3 *
R x SD	6	259.1		
Cultivar (C)	14	52.5	11.8	4.4 *
C x SD	28	12.4	11.8	1.1 ns
residual	126	11.8		
total	179			
		Edmonton (1988)		
Replication (R)	3	20.0		
Seeding Date (SD)	2	2914.2	78.4	37.2 *
R x SD	6	78.4		
Cultivar (C)	14	9.7	4.6	2.1 *
C x SD	28	3.4	4.6	<1.0 ns
residual	126	4.6		
total	179			
		Ellerslie (1988)		
Replication (R)	3	107.8		
Seeding Date (SD)	2	3474.1	99.1	35.1 *
R x SD	6	99.1		
Cultivar (C)	14	25.6	10.0	2.6 *
C x SD	28	8.2	10.0	<1.0 ns
residual	126	10.0		
total	179			

* = significant at P=0.05; ns = not significant.

Table 4.13a: Shoot dry weight (ShDW) means (g/10 plants) for 15 six-row barley cultivars grown at 2 central Alberta locations at different seeding dates, sampled 3 weeks after seeding. Means within columns followed by the same letter are not significantly different (P=0.05).

	Edmonton (1987)			Ellerslie (1987)		
Seeding Date	April 22	May 6	May 27	April 29	May 13	June 3
Conquest	0.53 abc	1.11 abc	3.54 ab	1.08 ab	1.46 ab	4.23
Bonanza	0.55 ab	0.99 abc	3.54 ab	0.85 abc	1.35 ab	4.10
Argyle	0.48 bcd	1.04 abc	2.75 b	0.43 c	1.15 b	4.07
Otal	0.59 a	1.04 abc	3.95 ab	1.22 a	1.45 ab	4.80
BT 522	0.51 bc	1.26 a	3.96 ab	1.25 a	1.37 ab	4.47
Heartland	0.45 cde	1.11 abc	3.96 ab	0.55 bc	1.41 ab	4.60
Klondike	0.46 bcde	1.07 abc	3.71 ab	0.77 abc	1.30 ab	4.40
BT 518	0.47 bcd	0.92 bc	3.29 ab	0.68 abc	1.33 ab	3.83
Johnston	0.54 ab	1.10 abc	3.98 ab	1.10 ab	1.67 a	4.53
Jackson	0.62 a	1.20 ab	4.86 a	1.23 a	1.61 ab	4.80
Leduc	0.48 bcd	0.95 bc	3.40 ab	0.81 abc	1.19 ab	4.20
Galt	0.41 de	0.86 c	3.48 ab	0.63 bc	1.21 ab	3.97
Diamond	0.47 bcd	0.92 bc	3.61 ab	0.98 abc	1.57 ab	3.97
Empress	0.51 bc	1.12 abc	3.74 ab	0.93 abc	1.38 ab	4.10
Samson	0.38 e	0.88 c	2.58 b	0.45 c	1.22 ab	4.10
Mean	0.50	1.04	3.62	0.86	1.38	4.28
SE	0.018	0.056	0.340	0.114	0.096	0.225
HSD	0.09	0.28	1.73	0.58	0.49	ns
CV	7.2%	10.8%	18.8%	26.5%	13.9%	10.5%

	Edmonton (1988)			Ellerslie (1988)		
Seeding Date	April 14	April 29	May 11	April 21	May 4	May 19
Conquest	0.39 a	0.62	2.28	0.68 abc	1.18	2.69
Bonanza	0.33 ab	0.54	2.00	0.73 abc	1.20	2.46
Argyle	0.31 ab	0.45	1.90	0.63 bc	1.06	2.31
Otal	0.34 ab	0.54	2.10	0.61 bc	1.24	2.59
BT 522	0.40 a	0.57	2.07	0.83 a	1.38	2.99
Heartland	0.32 ab	0.56	2.06	0.72 abc	1.41	2.76
Klondike	0.33 ab	0.51	1.85	0.71 abc	1.50	3.08
BT 518	0.32 ab	0.54	1.92	0.71 abc	1.08	2.74
Johnston	0.40 a	0.58	2.14	0.78 ab	1.20	3.14
Jackson	0.41 a	0.62	2.29	0.72 abc	1.28	3.03
Leduc	0.28 b	0.61	1.91	0.72 abc	1.27	3.20
Galt	0.33 ab	0.48	1.75	0.67 ab	1.19	2.61
Diamond	0.41 a	0.56	2.06	0.71 abc	1.41	2.80
Empress	0.39 a	0.62	2.17	0.77 ab	1.26	2.80
Samson	0.25 b	0.53	1.93	0.59 c	1.14	2.27
Mean	0.35	0.55	2.04	0.70	1.25	2.76
SE	0.022	0.042	0.118	0.036	0.133	0.289
HSD	0.11	ns	ns	0.18	ns	ns
CV	12.8%	15.2%	11.6%	11.0%	21.2%	20.9%

SE and CV = standard error and coefficient of variability of means in columns.
HSD = Tukey's honestly significant difference (P=0.05) among means in columns (ns = no significant difference among means in columns).

Table 4.13b: Shoot dry weight (ShDW) means (g/10 plants) for 15 six-row barley cultivars grown at 2 central Alberta locations at different seeding dates, sampled 5 weeks after seeding. Means within columns followed by the same letter are not significantly different (P=0.05).

	Edmonton (1987)			Ellerslie (1987)		
Seeding Date	April 22	May 6	May 27	April 29	May 13	June 3
Conquest	7.85 a	10.45 ab	26.53 a	13.10 a	18.20 ab	26.55 ab
Bonanza	7.50 a	10.83 ab	18.56 abc	11.10 abc	21.70 ab	25.58 ab
Argyle	3.85 de	10.18 ab	16.94 bc	8.90 bcd	16.13 ab	21.33 ab
Otal	5.00 abcde	10.93 ab	21.38 abc	11.90 abc	24.67 a	27.08 ab
BT 522	7.65 a	10.68 ab	24.31 ab	11.46 abcd	17.57 ab	26.70 ab
Heartland	6.87 abc	10.53 ab	19.97 abc	9.34 abcd	18.03 ab	26.85 ab
Klondike	6.45 abcd	12.05 a	18.25 abc	12.80 a	13.83 b	23.50 ab
BT 518	4.05 cde	10.03 ab	16.94 bc	7.90 d	15.23 b	18.90 b
Johnston	6.15 abcde	11.20 ab	21.19 abc	12.12 ab	18.97 ab	29.48 a
Jackson	7.05 ab	12.00 a	24.03 ab	12.54 ab	18.77 ab	27.68 ab
Leduc	4.40 bcde	10.33 ab	18.34 abc	10.30 abcd	18.13 ab	26.38 ab
Galt	4.51 bcde	10.40 ab	19.66 abc	8.70 bcd	16.03 ab	22.60 ab
Diamond	5.55 abcde	10.95 ab	20.34 abc	9.50 abcd	17.17 ab	20.03 ab
Empress	5.35 abcde	11.03 ab	20.38 abc	10.52 abcd	17.87 ab	23.10 ab
Samson	3.45 e	7.95 b	13.19 c	8.20 cd	16.67 ab	22.38 ab
Mean	5.71	10.63	20.00	10.56	17.93	24.54
SE	0.57	0.73	1.69	0.77	1.72	2.02
HSD	2.85	3.72	8.62	3.85	8.77	10.15
CV	17.5%	13.7%	16.9%	17.3%	19.1%	17.2%

	Edmonton (1988)			Ellerslie (1988)		
Seeding Date	April 14	April 29	May 11	April 21	May 4	May 19
Conquest	3.80	9.05	19.54	6.19	10.84	22.60
Bonanza	3.03	7.07	16.09	6.89	10.49	22.84
Argyle	2.89	9.37	16.70	5.30	10.67	18.03
Otal	3.01	9.02	17.51	6.38	10.85	18.97
BT 522	3.14	9.77	17.46	6.38	15.29	21.97
Heartland	3.03	9.72	17.39	6.16	14.87	23.39
Klondike	2.81	9.56	18.12	6.51	9.18	22.09
BT 518	2.69	8.83	14.77	5.95	10.23	18.73
Johnston	3.17	10.70	17.87	6.65	12.57	23.85
Jackson	3.61	9.56	17.45	7.50	15.39	23.65
Leduc	2.88	8.92	17.72	7.16	11.26	20.80
Galt	2.90	7.29	14.88	6.59	10.18	20.18
Diamond	4.17	10.55	17.87	6.02	12.81	21.30
Empress	3.19	10.50	19.15	6.65	14.49	25.70
Samson	2.75	8.71	13.18	5.16	10.72	17.31
Mean	3.14	9.24	17.04	6.36	11.99	21.43
SE	0.42	0.87	1.60	0.74	1.85	1.88
HSD	ns	ns	ns	ns	ns	ns
CV	26.8%	18.8%	18.6%	23.4%	30.9%	17.6%

SE and CV = standard error and coefficient of variability of means in columns.
HSD = Tukey's honestly significant difference (P=0.05) among means in columns (ns = no significant difference among means in columns).

However, these differences in ShDW cannot be attributed solely to differences in germination rates. Some cultivars, such as Conquest, rank high in both characteristics while others, like Samson, rank low in both characteristics. Most other cultivars that rank either high or low in one characteristic tend to rank mid-range in the other. For example, Jackson and BT 522 often rank high in growth rate and mid-range in germination rate. Argyle is an example of a cultivar that ranks at the extreme opposites for the two characteristics: high in germination rate and low in seedling growth rate. Lafond and Baker (1986) studied the germination rate, emergence rate and shoot dry weight of nine spring wheat cultivars in growth chambers over a range of temperatures (5 to 30°cultivar), and in field trials at a series of seeding dates. They reported significant cultivar differences in all three of these characteristics, and that cultivar ranking did not change at different temperatures or seeding dates. They also reported significant correlations between emergence rate and shoot dry weight for the combined data from three seeding dates, but when the same correlations were calculated on individual seeding dates many were not significant.

In the present study the field trial results confirm the conclusions drawn from the growth cabinet assays in Experiment 1. Genetic differences in both germination rate and seedling growth rate exist among the 15 test cultivars, and these two characteristics, both sub-components of vigor, occur in genotypes with some degree of independence from each other.

4.3: Experiment 3: Respiration Assays of Germinating Seeds

For rate of germination to be accepted by plant breeders as a genetically controlled vigor component that can be selected for, or at least monitored, in barley breeding programs, rapid and easy methodology for testing the GR of large numbers of individual genotypes is essential. All of the assay methods evaluated in this study so far involve substantial time and manpower commitments to obtain results from a relatively small number (15) of genotypes. In Experiment 3 two respiration rate assay methods are evaluated as possible indices for GR and seedling growth rate.

The tetrazolium reduction (TZR) method was adapted from a previously published study (Johnston et al., 1986) in which wheat genotypes were screened for differences in vigor by quantifying the ability of germinating seeds to reduce tetrazolium chloride via the respiratory metabolism. In the study by Johnston et al. (1986) the imbibed wheat seeds were manually dissected and only the embryos were tested. With barley this would be a near impossible task to perform with accuracy because of the tightly adhering husk (lemma and palea). In the present experiment whole seeds are used and the results are expressed as ratios with the dry weight of the seed batches included in the denominator.

The second method, CO_2 evolution (CDE) of germinating seeds, was chosen for a number of reasons. Previous studies have reported high correlation between respiration rate of imbibed seeds and germination rate or seedling vigor (Woodstock and Grabe, 1967; Kittock and Law, 1968). Respiration rate can be easily quantified by measuring CO_2 evolution, and the equipment to do this (a gas chromatograph) was available at the time the experiments were in progress. As an added feature of the CO_2 assay, it was possible to develop non-destructive procedures so that the same seed batches could then be used for TZR assays.

4.3.1 Carbon Dioxide Evolution

Analysis of variance of CDE data (Table 4.14) indicates significant temperature and cultivar main effects and a significant temperature X cultivar interaction. The seed source main effect and all interactions with seed source are not significant.

The cultivar mean CDE rankings, shown in Table 4.15, can be interpreted using the same procedure used to explain tables of means in Experiment 1. On the basis of the HSD intervals at the foot of each column any two means within a column are significantly different if their rank assignments are 6 to 8 positions apart. This is a rather broad generalization, but it holds true for the majority of comparisons and provides a quick way to compare cultivar ranking across assay temperatures and among experiments. Most notable from Table 4.15 are the ranking similarities with GR means (Table 4.2). The cultivars Conquest, Bonanza, Argyle and Otal all consistently rank high in CDE, as they did in germination rate (low GR). At the opposite end of the distribution Samson, Diamond and Leduc consistently rank low in both CDE and germination rate. As in all other experiments, the relative ranking of some cultivars changes with assay temperature but none consistently perform better or worse as temperature decreases.

4.3.2 Tetrazolium Chloride Reduction

Analysis of variance of TZR data (Table 4.16) produces results very similar to the CDE analysis. Temperature and cultivar main effects are significant and seed source is not significant. However, the seed source X cultivar X temperature interaction is significant, probably due to the behavior of a few cultivars. Most notable is Jackson which ranks high in the seed source 1 20°cultivar assay (Table 4.17) and mid to low in all other assays.

The ranking of cultivars by TZR (Table 4.17) is very similar to CDE rankings as well as to germination rate rankings from Experiment 1. However, one aspect of the TZR method appears to be different from the CDE method. The range of TZR values within the 5 and 10°cultivar assays is greater, in relation to HSD intervals, than the range within the 15 and 20°cultivar assays. Consequently, at the lower temperatures more significant differences among cultivars are detected, even though CV values are nearly the same.

To develop the methodology for these assays preliminary trials were conducted to determine appropriate time durations for seed imbibition and for exposure of the imbibed seeds to the tetrazolium chloride solution The preliminary tests indicated a necessity to reduce the time duration of both of these steps as assay temperature was increased in order to maintain the seeds in approximately the same growth stage (chitting) throughout the duration of each assay. The final procedure used to obtain the results reported here employed 24 hour time periods for imbibition and exposure to tetrazolium chloride for the 5 and 10°cultivar assays and 12 hour time periods for the 15 and 20°cultivar assays. The difference between the two protocols in detecting significant cultivar differences indicates a need for further testing and modification of procedures. Since the objective is to predict germination rate or emergence rate in the field, a procedure that detects cultivar differences similar to that which can be measured in growth cabinet assays or field trials would be most desirable.

Table 4.14: Analysis of variance of carbon dioxide evolution (CDE) data ($\mu mol\ g^{-1}\ h^{-1}$) for 15 six-row barley cultivars from 2 seed sources, assayed at 4 temperatures.

Source of Variation	Degrees of Freedom	Mean Square	Error Mean Square	F Ratio
Temperature (T)	3	467200	30920	15.1 *
Rep in Temp R(T)	12	30920		
Cultivar (C)	14	12400	458	27.1 *
C*T	42	1820	458	4.0 *
C*R(T)	168	458		
Seed Source (S)	1	13	4990	<1.0 ns
S*T	3	3890	4990	<1.0 ns
S*R(T)	12	4990		
S*C	14	412	424	<1.0 ns
S*C*T	42	430	424	1.1 ns
S*C*R(T)	168	424		
Total	479			

* = significant at P=0.05; ns = not significant.

Table 4.15: Carbon dioxide evolution index (CDE) means (μmol g^{-1} h^{-1}) for 15 six-row barley cultivars, from 2 seed sources, assayed at 4 temperatures. Bracketed numbers following CDE means indicate ranking within temperature.

Seed Source 1 (1985)

	20 °C	15 °C	10 °C	5 °C
Conquest	8.67 (2)	6.00 (2)	4.72 (3)	2.43 (3)
Bonanza	8.37 (3)	6.45 (1)	4.57 (4)	2.46 (2)
Argyle	8.84 (1)	5.64 (4)	5.17 (1)	2.50 (1)
Otal	7.54 (5)	5.50 (6)	4.34 (5)	2.21 (4)
BT 522	7.16 (8)	5.77 (3)	4.74 (2)	2.11 (6)
Heartland	7.84 (4)	5.43 (7)	4.01 (7)	1.98 (10)
Klondike	6.02 (11)	5.18 (8)	3.72 (9)	2.03 (9)
BT 518	6.14 (10)	4.78 (12)	3.63 (10)	1.53 (14)
Johnston	7.49 (6)	5.50 (5)	4.33 (6)	2.17 (5)
Jackson	7.37 (7)	4.59 (14)	3.89 (8)	2.07 (8)
Leduc	5.07 (14)	4.30 (15)	2.74 (14)	1.42 (15)
Galt	5.31 (12)	4.99 (10)	3.56 (11)	2.08 (7)
Diamond	5.17 (13)	5.00 (9)	3.53 (12)	1.88 (11)
Empress	6.33 (9)	4.76 (13)	2.70 (15)	1.81 (12)
Samson	4.97 (15)	4.87 (11)	3.34 (13)	1.64 (13)
Mean	6.82	5.25	3.93	2.02
SE	0.34	0.26	0.24	0.05
HSD	1.74	1.33	1.22	0.28
CV	10.0 %	9.9 %	12.3 %	5.5 %

Seed Source 2 (1986)

	20 °C	15 °C	10 °C	5 °C
Conquest	8.61 (2)	6.07 (4)	4.13 (3)	2.23 (5)
Bonanza	8.08 (4)	6.17 (3)	4.57 (1)	2.55 (3)
Argyle	8.57 (3)	6.40 (1)	3.58 (5)	2.57 (1)
Otal	7.20 (8)	6.34 (2)	3.59 (4)	2.48 (4)
BT 522	9.39 (1)	5.77 (5)	4.42 (2)	2.56 (2)
Heartland	7.57 (6)	5.71 (6)	3.41 (6)	2.20 (6)
Klondike	6.99 (9)	5.08 (11)	2.95 (12)	2.12 (8)
BT 518	5.46 (13)	4.82 (14)	3.03 (10)	1.85 (13)
Johnston	7.73 (5)	5.07 (12)	3.18 (7)	2.02 (10)
Jackson	7.57 (7)	5.08 (10)	3.06 (9)	2.09 (9)
Leduc	5.39 (14)	4.78 (15)	2.62 (15)	2.01 (11)
Galt	5.81 (10)	5.55 (7)	3.11 (8)	2.18 (7)
Diamond	5.15 (15)	5.37 (8)	2.89 (13)	2.01 (12)
Empress	5.50 (11)	5.34 (9)	3.00 (11)	1.75 (14)
Samson	5.46 (12)	4.96 (13)	2.78 (14)	1.74 (15)
Mean	6.96	5.50	3.35	2.16
SE	0.35	0.22	0.25	0.11
HSD	1.76	1.14	1.27	0.55
CV	9.9 %	8.1 %	14.8 %	10.1 %

SE and CV = standard error and coefficient of variability of means in columns.
HSD = Tukey's honestly significant difference (P=0.05) among means in columns (ns= no significant difference among means in columns).

Table 4.16: Analysis of variance of tetrazolium reduction (TZR) data ($\mu mol\ mol^{-1}\ g^{-1}$) for 15 six-row barley cultivars from 2 seed sources, assayed at 4 temperatures.

Source of Variation	Degrees of Freedom	Mean Square	Error Mean Square	F Ratio
Temperature (T)	3	41942.0	221.0	189.6 *
Rep in Temp R(T)	12	221.0		
Cultivar (C)	14	361.0	13.6	26.4 *
C*T	42	78.7	13.6	5.8 *
C*R(T)	168	13.6		
Seed Source (S)	1	183.0	58.4	3.1 ns
S*T	3	20.0	58.4	<1.0 ns
S*R(T)	12	58.4		
S*C	14	16.4	14.8	1.1 ns
S*C*T	42	25.0	14.8	1.7 *
S*C*R(T)	168	14.8		
Total	479			

* = significant at P=0.05; ns = not significant.

Table 4.17: Tetrazolium reduction (TZR) means (μmol mol^{-1} g^{-1}) for 15 six-row barley cultivars, from 2 seed sources, assayed at 4 temperatures. Bracketed numbers following TZR means indicate ranking within temperature.

Seed Source 1 (1985)

	20 °C	15 °C	10 °C	5 °C
Conquest	50.8 (1)	37.1 (1)	7.9 (6)	2.4 (4)
Bonanza	48.3 (2)	35.9 (3)	9.2 (3)	2.1 (5)
Argyle	46.0 (4)	33.2 (4)	8.7 (4)	2.7 (2)
Otal	45.0 (5)	36.6 (2)	12.5 (2)	3.4 (1)
BT 522	43.1 (6)	31.9 (5)	12.8 (1)	2.5 (3)
Heartland	42.9 (7)	29.9 (11)	5.9 (9)	1.1 (10)
Klondike	35.6 (10)	31.2 (7)	8.2 (5)	1.7 (6)
BT 518	38.8 (8)	29.6 (12)	5.3 (12)	0.7 (15)
Johnston	32.6 (11)	29.9 (10)	4.8 (13)	1.1 (9)
Jackson	47.0 (3)	30.2 (9)	6.5 (7)	0.9 (12)
Leduc	31.3 (13)	26.9 (14)	4.1 (14)	0.9 (11)
Galt	36.5 (9)	31.4 (6)	5.6 (10)	1.3 (7)
Diamond	30.5 (14)	28.5 (13)	5.6 (11)	0.7 (14)
Empress	31.8 (12)	30.2 (8)	6.0 (8)	1.1 (8)
Samson	28.2 (15)	26.7 (15)	4.1 (15)	0.8 (13)
Mean	39.2	31.3	7.1	1.6
SE	3.3	1.7	0.4	0.1
HSD	16.8	8.7	2.0	0.5
CV	17.0 %	10.7 %	11.4 %	15.0 %

Seed Source 2 (1986)

	20 °C	15 °C	10 °C	5 °C
Conquest	52.6 (3)	36.3 (5)	9.4 (4)	2.4 (5)
Bonanza	54.5 (1)	36.7 (3)	11.5 (2)	3.4 (2)
Argyle	45.7 (5)	38.7 (1)	11.0 (3)	2.3 (7)
Otal	54.0 (2)	36.4 (4)	8.3 (6)	3.7 (1)
BT 522	51.4 (4)	36.9 (2)	11.7 (1)	2.8 (3)
Heartland	42.9 (7)	29.6 (11)	8.7 (5)	2.3 (6)
Klondike	39.2 (10)	33.0 (7)	7.1 (9)	1.9 (11)
BT 518	28.4 (14)	28.6 (12)	5.7 (13)	1.9 (10)
Johnston	39.8 (9)	28.5 (13)	6.5 (10)	2.0 (9)
Jackson	40.5 (8)	33.4 (6)	8.1 (7)	2.2 (8)
Leduc	32.8 (13)	26.5 (14)	5.7 (12)	1.4 (15)
Galt	38.2 (11)	32.9 (8)	6.3 (11)	2.5 (4)
Diamond	37.2 (12)	26.0 (15)	7.6 (8)	1.8 (12)
Empress	43.9 (6)	32.4 (9)	5.2 (14)	1.6 (14)
Samson	25.8 (15)	30.4 (10)	4.7 (15)	1.7 (13)
Mean	41.8	32.4	7.8	2.3
SE	3.0	2.3	0.4	0.2
HSD	15.3	11.7	2.0	1.0
CV	14.3 %	14.0 %	10.4 %	18.8 %

SE.and CV = standard error and coefficient of variability of means in columns.
HSD. = Tukey's honestly significant difference (P=0.05) among means in columns (ns= no significant difference among means in columns).

4.4: Correlation Analysis Among Growth Chamber Assay, Field Trial, and Respiration Assay Results

In previous sections of this report many comparisons regarding relative cultivar rankings have been made, both within and among the different experiments. To test the statistical significance of association among the various assay techniques and field trial observations, Pearson correlation analysis is employed. In each correlation the pair of data sets used are the 15 cultivar means as presented in the various tables of means from the three experiments. All data sets are assumed to be measured with error and to vary independently of each other. In effect, the correlations test whether the variables can be considered covariates dependent on a common independent variable, genotype. A correlation is declared significantly different from zero (P=0.05) if the absolute value of the correlation coefficient (r) is greater than 0.51 (Sokal and Rohlf, 1980).

In Table 4.18 field observations from the three seeding dates are compared to growth chamber assays at 5, 10 and 20°C. These temperatures were chosen for correlation because they represent the full range of test temperatures and are common to all assays. For each pair of comparisons in the first column of Table 4.18 all three assay temperatures are tested with each of the three seeding dates. This is done since in all assays the cultivars ranked similarly, regardless of temperature.

Correlations of seed source 1 GR assays with DE in the field (1987 field trials) are all significant, but for seed source 2 GR correlations with 1988 field DE several individual r values are not all significant (Table 4.18). Random variability in emergence rate, caused by dry seedbed conditions in the 1988 field year, is most likely the cause of poor correlation with seed source 2 GR means in this case. The majority of the correlation coefficients for seed source 1 GR assays with ShDW from 1987 field plots are non-significant, and the correlation pairs that are significant seem to occur

Table 4.18: Growth chamber assay and field trial Pearson correlation values (r). Correlations are significantly different from zero when -.51> r >.51. Significant correlations are indicated in the table by bold face.

Seeding Date	1st			2nd			3rd		
Assay Temperature (°C)	5	10	20	5	10	20	5	10	20
Seed Source 1									
GR *with*									
- DE (Edmonton)	**.86**	**.81**	**.87**	**.81**	**.61**	**.92**	**.92**	**.69**	**.78**
- DE (Ellerslie)	**.79**	**.65**	**.67**	**.84**	**.73**	**.75**	**.86**	**.72**	**.78**
GR *with*									
- ShDW-3wk (Edmonton)	**-.67**	-.48	-.42	**-.64**	**-.56**	-.41	-.46	-.33	-.27
- ShDW-3wk (Ellerslie)	-.50	-.35	-.28	-.39	-.20	-.08	**-.54**	**-.52**	-.27
GR *with*									
- ShDW-5wk (Edmonton)	**-.60**	-.46	**-.55**	**-.56**	**-.56**	-.46	**-.62**	-.34	**-.52**
- ShDW-5wk (Ellerslie)	**-.64**	**-.73**	-.47	**-.58**	-.30	-.34	-.50	-.46	-.37
TDW *with*									
- ShDW-3wk (Edmonton)	.32	.31	**.68**	**.68**	**.63**	**.73**	.42	.40	**.74**
- ShDW-3wk (Ellerslie)	**.65**	**.52**	**.68**	.47	.43	**.77**	.31	.21	.41
TDW *with*									
- ShDW-5wk (Edmonton)	**.68**	**.51**	**.76**	.28	.26	**.62**	**.53**	**.52**	**.81**
- ShDW-5wk (Ellerslie)	**.52**	.36	**.55**	.30	.09	.15	**.52**	**.52**	.47
Seed Source 2 *									
GR *with*									
- DE (Edmonton)	.45	**.55**	**.75**	.43	.19	.35	**.81**	**.60**	.38
- DE (Ellerslie)	**.84**	**.81**	**.76**	**.61**	**.54**	**.53**	.44	**.61**	**.61**
TDW *with*									
- ShDW-3wk (Edmonton)	**.73**	**.61**	**.67**	.45	.40	**.56**	**.58**	.42	**.61**
- ShDW-3wk (Ellerslie)	**.71**	**.66**	**.60**	.33	.31	**.61**	.44	**.52**	**.65**
TDW *with*									
- ShDW-5wk (Edmonton)	.39	.31	**.60**	**.53**	**.71**	**.57**	**.65**	**.61**	**.76**
- ShDW-5wk (Ellerslie)	.33	.23	**.50**	**.50**	.46	.49	**.73**	**.73**	**.66**

* All seed source 2 GR *with* ShDW correlations are not significant.

at random with no meaningful trend with respect to either assay temperature of seeding date. All seed source 2 GR with 1988 field ShDW are non-significant and the r values are omitted from Table 4.18. Within the context of this study, germination rate measured in the growth cabinet reliably predicts cultivar differences in field emergence if moisture for germination is not limiting, but is not a reliable indice for seedling growth rate.

The reliability of growth chamber assays of seedling TDW in predicting ShDW of seedlings in the field is also marginal. Many of the r values for this comparison, for both 3 week and 5 week sampling times (Table 4.18), are not significant. Seedling TDW assays have two major limitations in relation to field conditions. First, growing conditions in the growth cabinet are fixed and do not duplicate fluctuations in light intensity, temperature or moisture availability that occur in the field. Cultivar differences in response to varying environmental conditions can be expected and would not be measured in controlled environment growth chambers. Second, TDW assays include root growth, which cannot be measured in the field. Cultivar differences in root-shoot partitioning may bias comparisons between field data and growth chamber data.

The correlations in Table 4.19 test the association among growth chamber and respiration assays. GR with TDW correlations at all temperatures are not significant. This supports the previous conclusion that these two characteristics vary independently of each other among cultivars. CDE and TZR are significantly correlated with each other at all temperatures, and both are significantly correlated with GR. In Table 4.20 CDE and TZR are compared to field emergence. All correlations of seed source 1 assay results with 1987 field DE are significant with the exception of one (TZR at 20°C with DE at Ellerslie, date 1). For seed source 2 comparison with 1988 field DE many correlations are not significant, again reflecting the variable emergence patterns during the 1988 field year. Both CDE and TZR reliably predict

cultivar differences in GR and field emergence and could be used as indices to screen cultivars on the basis of germination rate.

Table 4.19: Respiration assay and growth chamber assay Pearson correlation values (r). Correlations are significantly different from zero when -.51> r >.51. Significant correlations are indicated in the table by bold face.

	Seed Source 1				Seed Source 2			
Temperature (°C)	5	10	15	20	5	10	15	20
GR *with* TDW	-.34	-.12	.49	-.38	-.37	.29	-.23	-.08
GR *with* CDE	**-.82**	**-.56**	**-.70**	**-.79**	**-.74**	**-.74**	**-.72**	**-.71**
GR *with* TZR	**-.74**	**-.52**	**-.68**	**-.84**	**-.61**	**-.71**	**-.72**	**-.68**
TDW *with* CDE	.22	.15	-.11	.44	.18	-.01	.39	.41
TDW *with* TZR	.15	.05	-.29	.38	.11	-.05	.16	.41
CDE *with* TRZ	**.72**	**.65**	**.78**	**.85**	**.80**	**.87**	**.79**	**.75**

Table 4.20: Respiration assay and field trial Pearson correlation values (r). Correlations are significantly different from zero when -.51> r >.51. Significant correlations are indicated in the table by bold face.

Seeding Date	1st			2nd			3rd		
Assay Temperature (°C)	5	10	20	5	10	20	5	10	20
Seed Source 1									
CDE *with*									
- DE (Edmonton)	**-.82**	**-.66**	**-.77**	**-.64**	**-.55**	**-.71**	**-.81**	**-.69**	**-.82**
- DE (Ellerslie)	**-.64**	**-.56**	**-.70**	**-.58**	**-.52**	**-.71**	**-.68**	**-.55**	**-.76**
TZR *with*									
- DE (Edmonton)	**-.67**	**-.59**	**-.77**	**-.66**	**-.59**	**-.75**	**-.76**	**-.72**	**-.83**
- DE (Ellerslie)	**-.70**	**-.67**	-.44	**-.58**	**-.69**	**-.62**	**-.61**	**-.59**	**-.61**
Seed Source 2									
CDE *with*									
- DE (Edmonton)	**-.63**	-.47	**-.57**	-.16	-.22	-.22	-.47	**-.52**	**-.72**
- DE (Ellerslie)	**-.68**	**-.73**	**-.70**	**-.70**	**-.60**	**-.66**	**-.57**	-.36	-.40
TZR *with*									
- DE (Edmonton)	**-.60**	-.51	**-.64**	-.11	-.29	-.16	-.39	**-.55**	**-.61**
- DE (Ellerslie)	**-.51**	**-.71**	**-.75**	-.47	**-.69**	**-.85**	**-.57**	-.35	**-.61**

PART V: GENERAL DISCUSSION AND CONCLUSIONS

The results from growth chamber experiments and field trials conducted in this study demonstrate that there is significant variability among the 15 test cultivars in both germination rate and seedling growth rate. Also demonstrated is that these two characteristics, both vigor components, vary among genotypes with some degree of independence from each other. For example, some cultivars are characterized by high germination rate and seedling growth rate, while others are high in one attribute and low in the other. The basis for independence of germination rate and growth rate within a genotype is likely linked to the various growth and development processes associated with developing seedlings. From sowing to established stand, three distinct plant developmental stages can be delineated on the basis of predominating physiological processes.

During the first stage imbibition of water by the dormant seed begins, dehydrated tissues resume functional integrity, and respiration rate accelerates from the basal maintenance level. The rate limiting physiological processes during this stage are likely to be non-metabolic events, such as water diffusion through the husk, seed coat and cell membranes and the swelling of tissues and organelles. The potential for genetic variability in rate of development through this early stage is probably very limited among cultivars with very similar seed morphological characteristics, as are those used in this study. Seed size has been shown to affect germination rate (Boyd et al., 1971; LaFond and Baker, 1986a) with smaller seeds germinating faster. However, when seed size differences were removed experimentally, significant cultivar differences in germination rate were still present. Further, LaFond and Baker (1986b) were unable to detect differences in rate of water uptake due to cultivar or seed size effects. In the present study correlations

between cultivar mean kernel weight, an indication of seed size, and germination resistance were non-significant at all assay temperatures.

The second seedling development stage begins when embryo metabolic rate accelerates, and cell division and enlargement result in the growth of root and shoot initials. The primary growth rate limiting processes during this stage are likely associated with respiratory activities for the production of the energy (ATP) needed in the catabolism, transport, and re-assembly of substrates for new growth. Barley genotype differences in respiration rate have been investigated in previous studies (McDaniel, 1969; Woodstock and Grabe, 1967) and found to be positively associated with germination rate and seedling vigor. McDaniel (1969) reported that heterosis of mitochondrial efficiency measured in barley hybrids corresponded to higher tissue respiration rate, germination rate, and seedling growth rate when compared to parent lines. Since respiration metabolism is closely associated with mitochondrial membranes, low temperature stress that is severe enough to disrupt membrane integrity, as with lipid phase transition (Raison et al., 1971), would also be expected to have a large negative effect on respiration rate .

The third development phase begins after seedling emergence when the first leaves have developed chloroplasts and the plant loses its dependence on seed-stored energy reserves. During this stage, which extends to maturity, both respiration and photosynthesis are essential for energy production, and either may be the primary factor limiting growth rate. Both processes are membrane associated, but are located in different organelles, respiration in the mitochondria and photosynthesis in the thylakoids. Genotype differences in chlorophyll efficiency or concentration in leaf tissues could occur independently of differences in mitochondrial efficiency, and would also interact differently with environmental factors. For example, Wise and Naylor (1987) reported evidence of light induced superoxide damage to photosynthetic organelles in plants subjected to chilling stress. Damage caused by oxide radicals

occurs independently of the primary membrane phase change response to low temperature, and affects photosynthesis more than respiration.

In this study the primary difference between germination rate assays and seedling growth assays is the presence or absence of photosynthetic influences on the measured parameter. The germination resistance assays, CO_2 evolution and tetrazolium reduction, and field emergence rate measurements all assess cultivar differences in growth rate prior to the onset of photosynthesis. The results from these tests are significantly correlated to one another, indicating that genetic differences in respiration associated metabolic efficiency or capacity are primarily responsible for differences in germination and field emergence rate. Johnston et al. (1986) reported significant correlation between tetrazolium reduction by germinating wheat embryos and seedling growth in cabinets without light. The growth chamber plant dry weight assay and field trial shoot dry weight assay results are affected by both respiration and photosynthesis. Results obtained from these two tests were generally associated with respect to cultivar ranking, but many paired correlation tests were non-significant (Table 4.18). As seedling development progresses and photosynthesis becomes an influencing factor on plant growth, the increased influence of environmental variation masks genetic influences and weakens the correlation between growth chamber and field assays.

The results of this study confirm that significant genetic differences in germination rate and seedling growth rate exist among the 15 cultivars tested. These differences are significant at all growth temperatures within the range tested, and relative cultivar ranking does not change with temperatures. However, as growing temperature decreases the range among cultivars increases. In early seeded field plots, with low soil temperature (averaging 4°C), cultivar mean days to emergence ranged from 16.5 to 20.5, a difference of 4 days between the slowest and fastest emerging cultivars. In later seeded plots, with higher soil temperatures (averaging 16°C), the

range in time to emergence is as low as 2.0 days (7.0 to 9.0 days). Consequently, cultivar differences in emergence rate are much more noticeable, and may have more agronomic importance, under low temperature stress conditions than under normal conditions.

As mentioned in the introduction, this study was partially motivated by the apparent slow emergence and stand establishment characteristics of the cultivar Samson in comparison to other cultivars in agronomic field trials (Briggs, 1988). Of the 15 cultivars tested in this study, Samson is unique in that it consistently ranks low in both germination rate and seedling growth rate. It is likely that the combined effects of low growth and development rates at both the germination and early seedling stages are responsible for Samson's obviously inferior establishment capability. Samson is also the only semidwarf cultivar included in this study. Poor stand establishment, due to short coleoptile length and slow seedling growth, is a characteristic commonly associated with the semidwarf trait in cereal crop species (Allan et al., 1965). However, Bacaltchuk and Ullrich (1983) field tested a number of semidwarf and tall barley genotypes for stand establishment characteristics, and found that emergence rate and coleoptile length differences among semidwarf cultivars corresponded to the source of dwarfing genes in their pedigree. Cultivars with dwarfing genes from the Jotun line, which is also the source of dwarfism in Samson (Helm et al., 1986), often displayed poor establishment characteristics. In a study of semidwarf wheat genotypes, Allan (1980) concluded that the dwarfing genes in his study either reduce stand establishment capability themselves, or are closely linked to other genes that have negative effects. He further noted that the negative effects of the dwarfing genes on stand establishment can be reduced by backcrossing with certain non-dwarf parental lines.

Semidwarf cultivars are highly desirable because of their extreme lodging resistance, and future development of these types warrants high priority with barley

breeders in Western Canada. It should be noted that even though Samson is generally the slowest to established in field plots, it is often one of the highest yielding cultivars when lodging stress is severe (see yield data in Appendix A). Specific information concerning inherent germination rate and seedling growth rate characteristics of cultivars will aid breeders in choosing parental lines with superior stand establishment characteristics, and equally as important, will enable breeders to avoid lines with poor establishment performance. Also, with the current trends toward adoption of conservation tillage cropping systems, under which seedbed conditions are often less favorable for germination and emergence, producers would be better equipped to choose cultivars that are best adapted to specific situations if relative ratings for germination and emergence rate were available for commercial cultivars.

The CO_2 evolution and tetrazolium reduction assay procedures evaluated in this study would be useful techniques for screening genotypes in breeding programs, and for predicting stand establishment capabilities of cultivars under low temperature stress. Both of these methods produce indices that correlate to germination rate and field emergence rate assays, and require much less time and manpower commitment. Experimental errors (indicated by CV values in tables of means) are larger with the respiration assay methods when compared to germination tests, but the cultivar differences detected are sufficient to distinguish the extreme superior and inferior behaving cultivars. The respiration indices have the added advantage of being equally convenient to conduct at low or high temperatures. While germination resistance assays at 5°C take 2 to 3 weeks to complete, respiration assays of germinating seeds at 5°C can be completed in 2 to 3 days. Relative ranking of the cultivars included in this study does not change with temperature, but at lower temperatures cultivar differences are accentuated and easier to detect. If respiration indices are used to screen genotypes for selection in breeding programs, assays

conducted at both low and high temperatures (e.g. 5°C and 15°C) should detect genotypes displaying crossover interaction with temperature. This could potentially lead to the development of cultivars with specific tolerance to low temperature stress during germination and emergence.

Germination rate, field emergence rate and respiration rate indices of 15 barley cultivars are shown in this study to be significantly correlated to one another and relatively consistent over a range of assay temperatures and field conditions. Cultivar differences in seedling growth rate, assessed as plant dry matter accumulation, are significant, but are also much more susceptible to error due to environmental influences. For seedling growth rate to become a useful selection tool for plant breeders, more reliable assay methods need to be developed. Possibly a direct measurement of photosynthetic efficiency, such as chlorophyll florescences, would provide useful results.

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Appendix A: Grain yield (t/ha) for15 six-row barley cultivars grown at two central Alberta locations at different seeding dates. Grain samples were dried to approximately 9% moisture content before weighing.

Site (Year)	Edmonton (1987)			Ellerslie (1987)		
Seeding Date	April 22	May 6	May 27	April 29	May 13	June 3
Conquest	4.3	2.2	3.9	4.6	3.3	3.6
Bonanza	4.7	2.7	3.6	4.9	3.6	3.4
Argyle	4.7	2.8	4.0	4.8	4.1	4.3
Otal	3.8	3.8	3.5	3.5	3.2	3.1
BT522	4.3	2.9	3.2	3.8	3.5	2.9
Heartland	4.8	3.6	4.1	3.6	2.9	3.2
Klondike	4.6	2.9	3.8	4.8	3.8	3.8
BT 518	5.1	3.9	4.1	3.1	3.5	3.0
Johnston	4.8	4.3	4.1	4.6	4.1	3.9
Jackson	4.7	3.7	4.0	5.1	4.2	3.5
Leduc	4.6	3.6	3.4	3.5	3.8	2.7
Galt	5.1	3.0	3.9	4.7	3.4	3.1
Diamond	5.2	3.6	3.9	3.7	3.8	3.4
Empress	4.4	3.2	3.0	5.0	4.1	3.8
Samson	4.3	3.0	4.3	4.8	4.6	3.9
Mean	4.6	3.3	3.8	4.3	3.7	3.4
S.E.	0.21	0.22	0.29	0.32	0.35	0.29
C.V.	8.9%	13.0%	15.4%	11.2%	14.8%	10.6%

Site (Year)	Edmonton (1988)			Ellerslie (1988)		
Seeding Date	April 14	April 29	May 11	April 21	May 4	May 19
Conquest	4.7	3.8	3.4	5.0	4.1	3.2
Bonanza	4.8	4.3	3.5	4.9	3.9	3.5
Argyle	5.2	3.5	3.7	5.0	3.5	3.6
Otal	2.8	2.6	2.3	3.8	3.4	2.1
BT 522	3.8	3.2	2.8	4.4	3.3	4.0
Heartland	4.5	3.6	3.6	4.2	3.7	3.6
Klondike	4.4	2.9	3.3	4.4	3.3	3.7
BT 518	4.7	3.7	2.5	4.3	3.8	3.3
Johnston	3.4	2.8	2.5	4.7	3.7	2.8
Jackson	4.5	3.6	2.7	5.2	3.9	2.5
Leduc	3.7	3.7	3.1	4.2	3.9	3.8
Galt	4.3	3.9	3.0	4.8	4.0	3.2
Diamond	4.5	3.0	2.1	4.5	3.5	2.5
Empress	4.3	3.7	3.2	4.5	3.0	3.2
Samson	4.9	4.5	5.4	5.2	5.1	4.6
Mean	4.3	3.5	3.1	4.6	3.7	3.3
S.E.	0.48	0.34	0.30	0.36	0.42	0.35
C.V.	22.3%	19.4%	19.2%	15.5%	22.3%	21.4%

S.E. and C.V. = standard error and coefficient of variability of means in columns.

Appendix B: Lodging indices (means of 4 replications) at harvest time for15 six-row barley cultivars grown at two central Alberta locations at different seeding dates. Individual treatments were assessed visually and assigned a rating between 0 (no sign of lodging) and 9 (totally lodged).

Site (Year)	Edmonton (1987)			Ellerslie (1987)		
Seeding Date	April 22	May 6	May 27	April 29	May 13	June 3
Conquest	4	6	2	7	3	4
Bonanza	6	7	1	8	5	2
Argyle	7	5	0	7	3	3
Otal	3	5	5	8	6	3
BT522	5	6	5	7	7	4
Heartland	6	6	1	7	2	2
Klondike	6	8	0	8	4	2
BT 518	5	7	3	7	4	2
Johnston	7	9	8	9	7	6
Jackson	4	7	2	6	3	3
Leduc	6	9	6	8	6	5
Galt	1	6	0	6	4	1
Diamond	3	5	1	6	0	4
Empress	6	7	3	7	5	3
Samson	2	4	0	6	0	0

Site (Year)	Edmonton (1988)			Ellerslie (1988)		
Seeding Date	April 14	April 29	May 11	April 21	May 4	May 19
Conquest	8	8	7	4	3	7
Bonanza	8	6	9	5	8	7
Argyle	8	9	8	6	7	2
Otal	9	9	8	4	9	9
BT 522	9	9	9	5	9	8
Heartland	7	8	8	5	7	9
Klondike	8	9	8	7	8	6
BT 518	9	9	9	7	9	8
Johnston	9	9	9	7	6	7
Jackson	9	9	9	4	5	9
Leduc	8	8	9	7	9	9
Galt	8	9	9	7	7	9
Diamond	9	7	9	5	8	9
Empress	9	9	8	6	9	9
Samson	5	4	2	2	3	1